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NOTES ON TROMBICULID MITES WITH DESCRIPTIONS OF WALCHIINAE N. SUBF., *SPEOTROMBICULA* N. G., AND *EUTROMBICULA DEFECTA* N. SP.

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Included in the following notes on mites of the family Trombiculidae are a key to the subfamilies, the redescriptions of two old genera, the description of a new subfamily based on larval characters, of a new American genus based on adult characters, and a new American species based on larval characters.

The Family Trombiculidae

The family Trombiculidae as established (Ewing, 1944) included two subfamilies, the Hemitrombiculinae and the Trombiculinae. To these Womersley (1944), added another, the Leeuwenhoekiinae, which included at the time it was erected only the single genus *Leeuwenhoekia* Oudemans, 1911. Later (1945), he erected the family Leeuwenhoekiidae Womersley, which included several genera. As here used the subfamily Leeuwenhoekiinae includes six genera in which there is a pair of submedian setae present on the dorsal plate. They are given in the writer's key to the genera (Ewing, 1945).

KEY TO THE SUBFAMILIES OF TROMBICULIDAE BASED ON LARVAL CHARACTERS

A. Dorsal plate with either a median seta or a pair of submedian setae on or near anterior margin and frequently with an anterior median process; tracheae and spiracles frequently present.
  B. Dorsal plate with only a median seta on or near anterior margin and very seldom with an anterior median process; tracheae and spiracles rarely present ..... Trombiculinae Ewing
  BB. Dorsal plate with a pair of submedian setae on or near anterior margin and usually with an anterior median process; tracheae and spiracles usually present ..... Leeuwenhoekiinae Womersley
AA. Dorsal plate without either a median or submedian setae, and without anterior median process; tracheae and spiracles absent.
  B. Pseudostigmatic organs represented by unmodified, simple setae; tarsi two-clawed; body setae simple ..... Hemitrombiculinae Ewing
  BB. Pseudostigmatic organs true sensillae; tarsi three-clawed but one or two claws frequently much reduced; body setae barbed or branched. Walchiinae, new subfamily

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WALCHIINAE, new subfamily

Characters for larval stage: Piercing part of chelicera, bladelike, upcurved, without a row of dorsal teeth and extending beyond apex of basal cheliceral segment. Tracheae and spiracles absent. Dorsal plate without median or submedian setae, frequently extending backward over part of dorsum of abdomen and bearing one or more pairs of dorsal, abdominal setae. Pseudostigmatic organs not simple setae, but modified into true sensillae. Abdomen frequently constricted near middle but never with a pair of posterodorsal plates. Legs rather short; tarsi three-clawed but one or two of the claws frequently much reduced.

Included genera: Walchia Ewing, *Gahrliepia* Oudemans, *Schöngastiella* Hirst, and *Gateria* Ewing.

A REDESCRIPTION OF THE GENUS *Odontacarus*

The genus *Odontacarus* Ewing was established many years ago for a species that was inadequately described. When the holotype of this species was prepared for study it was stained in acid fuchsin and mounted in balsam. Now, unfortunately, the specimen has lost its fuchsin stain and being mounted in balsam but few of its finer characters can properly be observed. However, by the aid of a microscope provided with Zeiss phase-contrast equipment and the assistance of Lieutenant G. W. Wharton, it has been possible to obtain some additional valuable information not only from the holotype, *O. dentatus,* but also from the other included species of *Odontacarus, O. australis* (Ewing). A redescription of *Odontacarus* is here given based on larval characters.

Odontacarus Ewing

1929. *Odontacarus* Ewing, Manual of external parasites, p. 188.

Characters for larval stage: Chelicera rather stout; piercing blade with a row of backwardly directed teeth above and one or more teeth below. Palpus medium to stout; palpal claw bifurcate or trifurcate. Tracheae and spiracles present. Dorsal plate without transverse or median crest and without circular striations but may possess an anterior median process. Setae on dorsal plate, exclusive of pseudostigmatic organs, are one median, a pair of anterolaterals and a pair of posterolaterals. Anterolaterals setiform, not stout and peglike. Pseudostigmatic organs flagelliform. Abdomen not constricted near middle, without posterodorsal plates. Legs rather slender; coxa I with 2 setae; coxa II and coxa III each with a single seta; tarsi three-clawed, middle claw more slender than other two which are equal.

Type species: Trombicula dentata Ewing. Other included species: *Odontacarus australis* (Ewing).

One of the species of *Odontacarus, O. australis,* shows clearly the presence of an anterior median process extending from the dorsal plate and a pair of spiracles in front of the anterior coxae, each spiracle being connected with a tracheal trunk. The type species is without the anterior median process, but is provided with tracheae and a pair of spiracles.

Odontacarus is related to *Acomatacarus* Ewing, 1942, from which it differs in having a single median seta on the dorsal plate instead of two submedian setae. It also is related to *Endotrombicula* Ewing, 1931, from which it may be separated by the following key:

Chelicera with more than 3 subequal dorsal teeth; tracheae and spiracles present; dorsal plate well developed, with or without anterior median process; median seta pectinate. Not living under surface of skin of hosts.

Odontacarus Ewing

Chelicera with 3 unequal dorsal teeth; tracheae and spiracles absent; dorsal plate vestigial; median seta simple. Living under surface of skin of amphibians.

Endotrombicula Ewing

A NEW GENUS OF AMERICAN TROMBICULIDS

The characters which are of value in describing the adult stage of trombiculids are not yet well known, hence generic descriptions based on them are to be regarded as somewhat preliminary at present. Such a description based on adult characters is here given.

Speotrombicula, new genus
(Fig. 1)

Characters for adult stage: Palpi slender, slightly curved; palpal claw acute at apex, with a single accessory spine near base; palpal patella not emarginate ventrally. Epistome a poorly sclerotized, bandlike comb. One rostral seta present. Eyes absent. Crista broad, platelike; pseudostigmata small, far apart; pseudostigmatic organs minute, setiform. Abdomen slightly constricted in front of middle. Dorsal abdominal setae barbed, of one kind, longest on posterior margin. Genital opening large, with 3 pairs of genital suckers. Coxa I with a shelflike expansion along its anterior margin. Coxa I and II united. Anterior legs much larger and longer than others and each ending in a pair of subequal, curved, distally trifurcate claws. Legs II, III, and IV nearly equal, weak and each ending in a pair of large, strongly curved, simple claws.
Type species: Trombicula trifurca Ewing, 1933.

When the original description of *trifurca* was written the epistome and rostral seta were overlooked. They have seldom been mentioned by others in any species of trombiculids, although they are of generic significance. The position of this genus is an open question. Presumably it belongs among those in which the eyes are absent. The larvae of the only contained species probably parasitize bats.

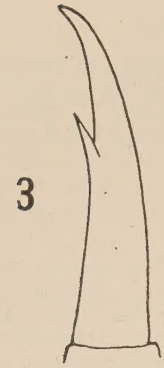
Pentagonella Thor, 1936 and *Neotrombicula* Hirst, 1925

These two derivatives of the genus *Trombicula* Berlese have not been recognized by most workers. The genus *Pentagonella* was established by Thor, 1936, with *Trombidium ardeae* Trägårdh as type. As proposed the genus corresponded to Oudemans's (1912) "*muris*-Gruppe of *Microthrombidium,* to which was added *Trombicula acuscutellaris* Walch and *T. yorkei* Sambon. The character on which this genus was based was the pentagonal shape of the dorsal plate. It should be noted in this connection that in the type species of *Neotrombicula* Hirst, 1925, *Microthrombidium autumnalis* Shaw, that the dorsal plate (Fig. 2) approaches a pentagonal shape. Furthermore, Wharton, 1945, has described a species of *Trombicula, T. frittsi,* which apparently does not belong to the "*muris*-Gruppe" yet has the pentagonal dorsal plate. The status of both *Pentagonella* and *Neotrombicula* will ultimately depend largely upon a study of the characters of the adult stage of the type species of each. The adult of the type species of *Pentagonella* is unknown. The characters of the adult of the type species of *Neotrombicula* make it generically distinct from the adult of the type species of *Trombicula.*

A REDESCRIPTION OF THE GENUS *Eutrombicula*

The genus *Eutrombicula* Ewing, 1938, was established with *Microthrombidium alfreddugèsi* Oudemans as type, and was based on larval characters. Later the genus was restricted by separating from it the genus *Acariscus* Ewing, 1943.

In considering the validity of *Eutrombicula,* adult characters should be regarded as of equal, if not more, importance than larval characters. Unfortunately, however, while the adult of the type species is known and is noted to be generically distinct, adults of most of the other species of the genus are unknown. Based on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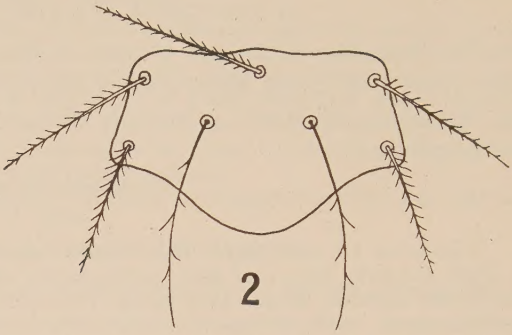
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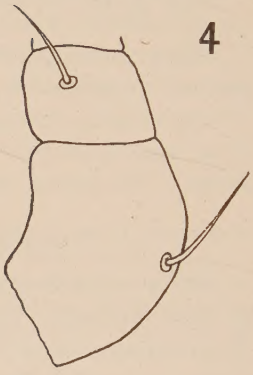
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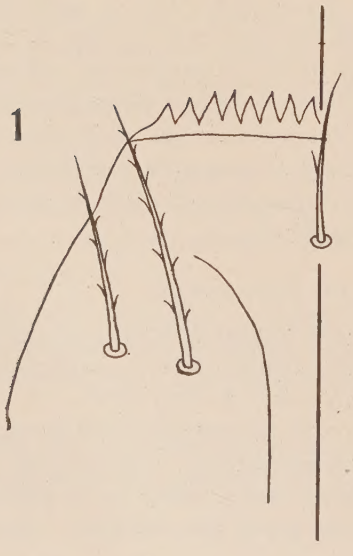
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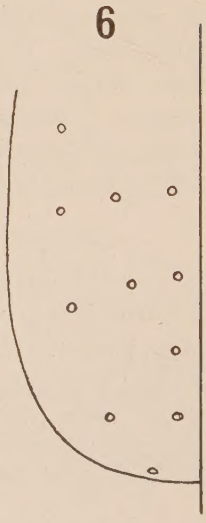
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what is known up to the present of the adult characters, *Eutrombicula* is nearest to *Acariscus* Ewing and *Trombiculoides* Jacot, 1938. The status of the last named genus is somewhat in doubt as it apparently was established for a misidentified species. According to the description and figures given by Michener (1946) of the adult of a species which he regarded as a synonym of the type species of *Acariscus, Trombicula flui* van Thiel, it differs from the adult of the type species of *Eutrombicula, E. alfreddugèsi* (Oudemans), in the absence of a median carina on the crista, the shape of the sunken area of the area sensilligera and in having eyes with unreduced corneas.

Eutrombicula Ewing

1938. *Eutrombicula* Ewing, Jour. Wash. Acad. Sc., vol. 28, p. 293.

Characters for the larval stage: Chelicera stout; piercing distal segment bladelike, upcurved, with a single tooth above and usually one below, and extending beyond apex of basal cheliceral segment. Palpus usually stout; palpal femur laterally angulate or rounded; palpal claw bifurcate. Tracheae and spiracles absent. Dorsal plate broader than long, without transverse or median crest and without circular striations, anterior margin about straight or slightly biconcave, posterior margin broadly and evenly outcurved. Pseudostigmatic organs flagelliform and usually with lateral barbs or branches. Setae on dorsal plate five; one barbed or branched median, a pair of barbed or branched, setiform anterolaterals, and a pair of posterolaterals similar to anterolaterals. Abdomen without posterodorsal plates; dorsal abdominal setae 22 (counting posterior marginals and dorsolaterals of row IV) and in unengorged larvae arranged as follows: 2–6–6–4–2–2; ventral body setae of first three rows with following arrangement: 2–2–6, the middle pair of setae of row III being situated farther forward than the other two pairs of the same row. Legs varying in length; coxae each with a plumose seta; tarsi three-clawed, the middle claw being longer and more slender than the other two which are equal.

Type species: Microthrombidium alfreddugèsi Oudemans, 1910.

The most important character in this genus is not the number of dorsal abdominal setae which is 22, but their definite arrangement in the 2–6–6–4–2–2 pattern in the unengorged larva, or 2–6–6–2–4–2 in most engorged larvae. Also correlated with this character there are two others, the arrangement of the six ventral setae of row III and the particular type of dorsal plate which has been described.

Eutrombicula defecta, new species
(Figs. 3, 4, 5, 6)

Characters for larval stage: Chelicera with swollen basal segment; piercing apparatus upcurved, with a single dorsal and apparently no ventral tooth. Palpus rather stout; palpal femur subangulate laterally; first palpal seta very slightly curved, simple and extending beyond the anterior margin of the palpal femur; second palpal seta straight, simple, extending for about one half its length beyond distal margin of patella; third palpal seta recurved, simple; inner prong of palpal claw very small, almost straight, sharply pointed; outer prong slender, curved. Galeal seta simple. Tracheae and spiracles absent. Dorsal plate not pitted, with anterior margin incurved on either side of median seta; sides slightly incurved, and posterior margin evenly and slightly outcurved; anterolateral seta curved, slightly barbed, when depressed backward reaching about to base of posterolateral; median seta longer than anterolateral and when depressed

EXPLANATION OF PLATE

The drawings here given are of different magnifications.

FIG. 1. Anterior part (left half) of cephalothorax of *Speotrombicula trifurca* (Ewing) showing the epistome and rostral seta.

FIG. 2. Dorsal plate of *Microthrombidium autumnalis* Shaw. (After André.)

FIG. 3. *Eutrombicula defecta* n. sp.; dorsal view of palpal claw.

FIG. 4. *Eutrombicula defecta* n. sp.; dorsal view of femur and patella of palpus.

FIG. 5. *Eutrombicula defecta* n. sp.; eyes and dorsal plate.

FIG. 6. *Eutrombicula defecta* n. sp.; diagram showing arrangement of seta pits of dorsal abdominal setae.

extending almost to posterior margin of dorsal plate; posterolateral seta similar to median and of about same length; pseudostigmata situated slightly behind middle of dorsal plate; pseudostigmatic organs slender, flagelliform, simple, and when depressed extending beyond posterior margin of dorsal plate by about one-half their length. Eyes without ocular plate, posterior eye the smaller and without cornea. Dorsal setae 2,6,6,2,4,2 in partly engorged specimens. Ventral setae apparently 2,2,6,4,2. A single, minutely barbed seta on each coxa. Tarsus I with a simple dorsal spine and a clavate dorsal spine. Tarsal armature as usual.

Length of dorsal plate, 0.047 mm; greatest width, 0.094 mm; length of partly engorged individual, 0.33 mm; width, 0.21 mm.

Type material: Two cotypes, U. S. National Museum No. 1429.

Type host: "Spiny rat."

Type locality: Erwin Island, Canal Zone.

Remarks.—Described from two specimens mounted on the type slide. They were taken from the type host at the type locality July 28, 1928, by Raymond Shannon.

This chigger is named *defecta* in reference to the degenerate or "defective" inner prong of the palpal claw, which character sets it apart from nearly all other species of its genus. Also the species is unusual in having not only the pseudostigmatic organs simple but likewise all of the first three palpal setae simple.

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TWO NEW SPECIES OF *TROMBICULA: T. MONTANENSIS* AND *T. APLODONTIAE* (ACARINA, TROMBICULIDAE) FROM NORTHWESTERN UNITED STATES[1]

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The two species described herein are placed tentatively within the genus *Trombicula, sens. lat.* Both species are distinctive, not only morphologically, but also with respect to their host relationships. Some taxonomists would doubtless consider that they represent new genera. However, until the taxonomy of the TROMBICULIDAE becomes more stabilized, it is the writer's opinion that a conservative attitude is desirable.

Trombicula montanensis n. sp.

(Fig. 1)

The larvae, in life, are small, broad oval, pale yellow.

Size: Derived from measurements, in microns, of 35 well engorged larvae. Length including capitulum: maximum 515, minimum 348; width: maximum 390, minimum 218. No unfed larvae were available.

Capitulum (Fig. 1, B): Chelicerae long, slender, moderately curved, in some specimens an inconspicuous accessory ventral tooth is present. Base of chelicera nearly as broad as long, lacking punctations. Seta of cheliceral shield nude or with one or two branches near its base. Palpi rounded to subangulate; the thumb longer than broad and with six branched setae, one nude seta and a sensory(?) spur; claw trifurcate, the median prong largest; palpal setae (not including the ventral plumose seta at the base) five, about equal in length, 1 and 2 with not more than six branches, 3 with one or two branches, 4 and 5 nude or with a branch near the base.

Scutum (Fig. 1, A): Slightly more than twice as wide as long with a broadly outcurved posterior margin and slightly incurved anterior margin; punctate as figured. All setae short, similar in form and approximately the same length, quite like the dorsal setae, their branches often somewhat appressed. Sensillae flagelliform with about ten fairly long branches along the distal half; sensillary bases separated by slightly more than the distance between anterolateral and posterolateral setae and located nearly midway between the anterior and posterior margins.

Standard measurements (Womersley and Heaslip, 1943 and Womersley, 1944) *for holotype:* AW–58, PW–83, AP–23, SB–25, ASB–26, PSB–21, AM–29, AL–30, PL–29, S– —, ADS–28, MDS–26, PDS–25. Average of ten specimens: AW–58, PW–86, AP–24, SB–28, ASB–25, PSB–22, AM–26, AL–26, PL–29, S–60, ADS–29, MDS–26, PDS–24.

Dorsum (Fig. 1, D): Eyes 2/2, within an ocular plate.

Dorsal formula: 2–6–6–6–2–2. Setae similar in form and size to the scutal setae, no extreme variation in length, but measurements show them to become slightly shorter from anterior to posterior rows.

Venter (Fig. 1, C): Setae similar to dorsal setae, but little shorter and thinner; rows not always uniform. Including the sternal setae, the ventral formula may be interpreted as: 2–2–8–8–6–2–2–2, however more allowance must be made for variation than in the dorsal formula. Anus relatively close to posterior margin of abdomen. Dependent on interpretation, only one pair of setae are posterior to the anus.

Legs: Typical for the genus. Tarsi I and II have a middorsal sensory(?) spur which is absent on tarsus III. Coxae (Fig. 1, E) I and II with one seta, III with either three or four setae.

Type data: Holotype: larva from *Cynomys ludovicianus ludovicianus* (black-tailed prairie dog), Wheatland County, Montana, August 30, 1945, AP–21982.

Paratypes: 27 larvae, same data as holotype; 22 larvae, same host species, locality and date, AP–21983; 5 larvae from *Sylvilagus nuttalli grangeri* (cottontail rabbit) same locality, August 31, 1945, AP–21984.

Collections made by personnel of the Rocky Mountain Laboratory.

Received for publication, July 3, 1946.

[1] From the Rocky Mountain Laboratory (Hamilton, Montana), National Institute of Health.

Holotype and 34 paratypes deposited in the collection of the Rocky Mountain Laboratory, 10 paratypes in the U. S. National Museum, 5 paratypes in the South Australian Museum, and 5 paratypes in the British Museum.

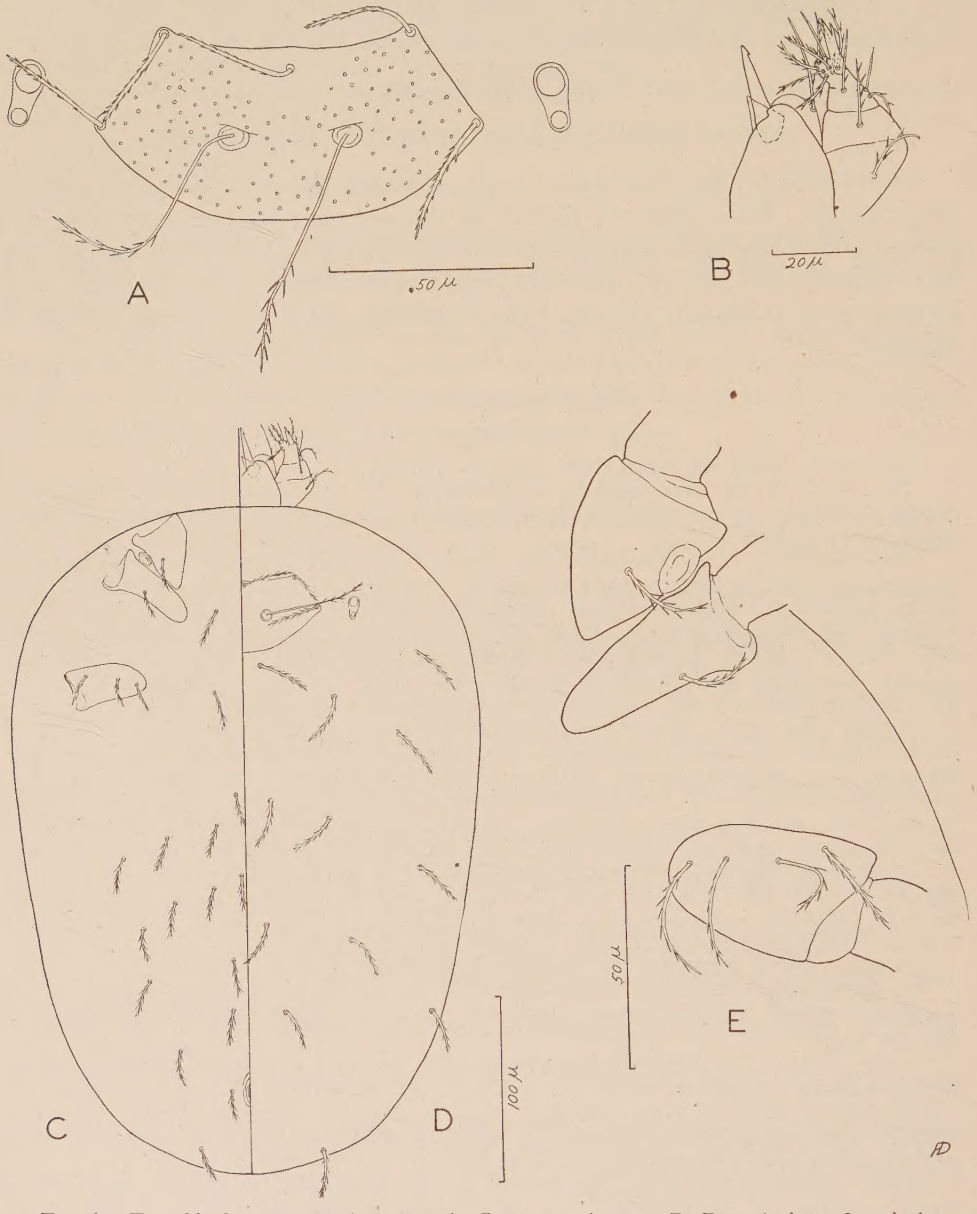

FIG. 1. *Trombicula montanensis* n. sp. A. Scutum and eyes. B. Dorsal view of capitulum. C. Venter. D. Dorsum. E. Coxae.

Comment.—From the collecting data, it is apparent that *Trombicula montanensis* n. sp. exhibits preference for prairie dogs. It is believed that the occurrence of this chigger on a cottontail in association with the prairie dog colony may be accidental.

The scutum and the 3 to 4-setose coxa III should readily separate *T. montanensis* from other North American chiggers of the group.

Trombicula aplodontiae n. sp.

(Fig. 2)

In life, the larvae are large, oval, brown yellow.

Size: Derived from measurements in microns of 40 larvae in varying degrees of engorgement. Length including capitulum: maximum 1056, minimum 307; width: maximum 764, minimum 198. Some of the smallest specimens measured were virtually unfed.

Capitulum (Fig. 1, D): Chelicerae moderately curved, with small subapical dorsal and ventral tooth. Base of chelicera longer than broad, the outer margin not evenly curved, the inner margin not conspicuously notched distally, punctate as figured. Seta of cheliceral shield plumose

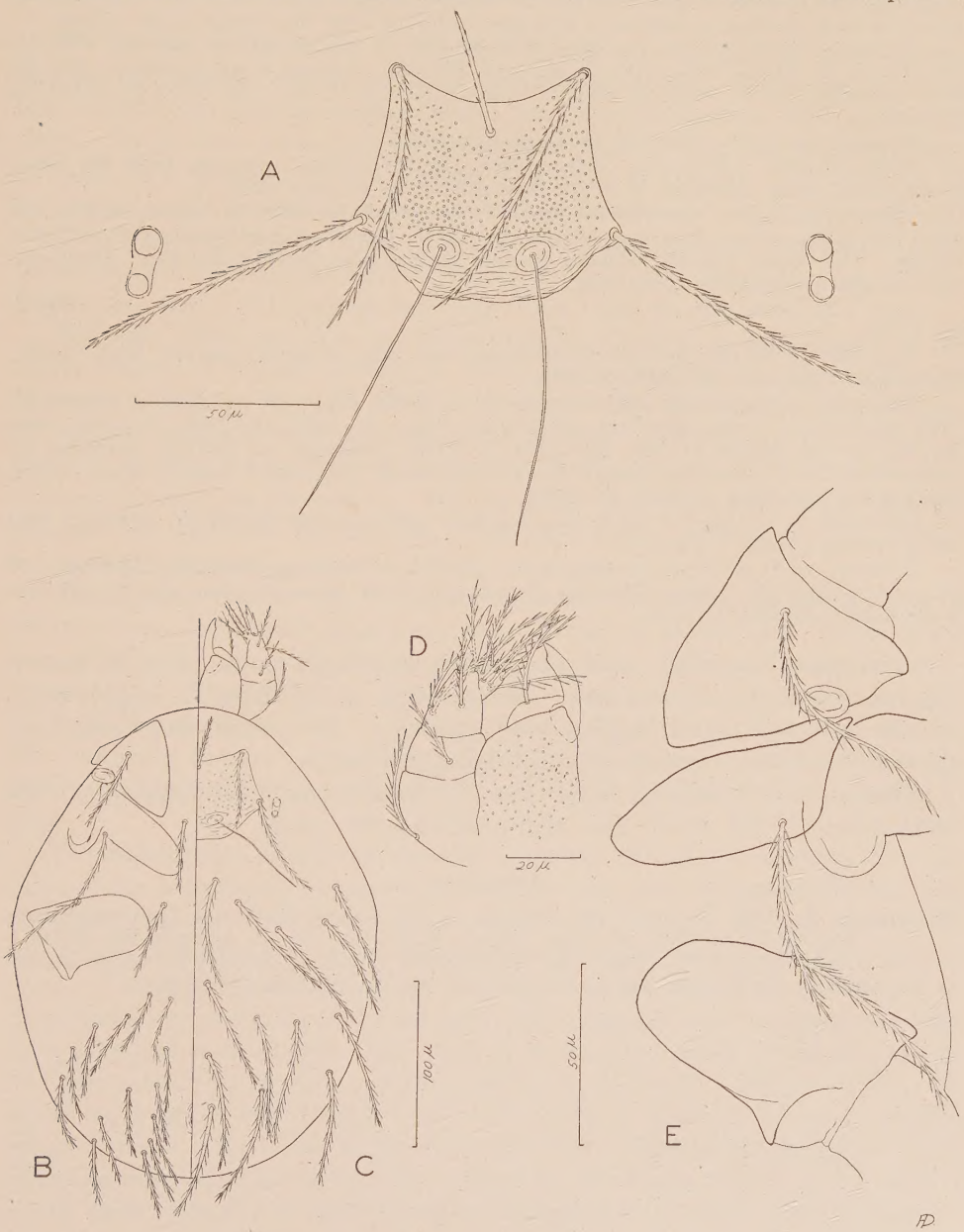

FIG. 2. *Trombicula aplodontiae* n. sp. A. Scutum and eyes. B. Venter. C. Dorsum. D. Dorsal view of capitulum. E. Coxae.

for its entire length. Palpi rounded; thumb robust, little longer than broad and with seven long plumose setae and a moderately long sensory(?) spine; claw trifurcate, the median prong longest; palpal setae (excluding the basal one) five, all plumose, 2 and 4 half the length of 3, 1 and 5 about equal length and a trifle shorter than 3.

Scutum (Fig. 2, A): But slightly broader than long, roughly hexagonal; anterior margin strongly incurved; the sinuous three-sided posterior margin defined only by a series of long transverse striations which extend into the area sensilligera; punctate as figured. All setae plumose, but the anteromedian with shortest and least branches; anteromedian, anterolaterals and posterolaterals increase in length respectively. Sensillae simple, flagelliform, their cup-like bases separated by slightly more than half the distance between anterolateral and posterolateral setae; the area sensilligera, because of striations, is distinct from the surface of the scutum.

Standard measurements for holotype: AW–50, PW–72, AP–40, SB–25, ASB–39, PSB–11, AM–40, AL–69, PL–84, S–76, ADS–79, MDS–69, PDS–47. Average of ten specimens: AW–49, PW–74, AP–42, SB–26, ASB–40, PSB–13, AM–41, AL–70, PL–83, S–82, ADS–79, MDS–71, PDS–55.

Dorsum (Fig. 2, C): Eyes 2/2, within an ocular plate.

Dorsal formula: 2–8–8–4–2–2. Setae plumose, similar in form, averaging twice the length of the ventral setae, decreasing in length from anterior to posterior rows.

Venter (Fig. 2, B): Ventral setae similar in form to dorsal setae, but slightly thinner and about half as long, arranged in irregular rows beyond the sternal setae and somewhat variable in number. The ventral formula including sternals approximates 2–2–10–6–8–2–2–2. About two rows of setae are posterior to the anus.

Legs: The dorsal spurs on tarsi I and II arise from the distal 1/3. Coxal setae (Fig. 2, E) 1–1–1.

Type data: Holotype: larva from *Aplodontia rufa rufa* (mountain beaver), King County, Washington, September 12, 1945, AP–21994.

Paratypes: 9 larvae, same data as holotype; 21 larvae, same host and locality, August 15, 1945, AP–21992; 5 larvae, same host and locality, August 26, 1945, AP–21991; 31 larvae, same host and locality, August 20, 1945, AP–21993; 19 larvae, same host and locality, September 15, 1945, AP–21986; 1 larva from *Mustela frenata altifrontalis* (long-tailed weasel), King County, Washington, September 12, 1945, AP–21996.

Collections by personnel of the Rocky Mountain Laboratory and George W. Schenk, a high school student, Bothell, Washington.

Holotype and 51 paratypes deposited in the collection of the Rocky Mountain Laboratory; 15 paratypes in the U. S. National Museum, 10 paratypes in the British Museum, and 10 paratypes in the South Australian Museum.

Comment.—Trombicula aplodontiae occurs abundantly on the mountain beaver. Preference is exhibited for the ears. The occurrence of this distinctive chigger on an equally distinctive rodent suggests host specificity. The weasel, from which one specimen was taken, was captured in an *Aplodontia* burrow.

This is the first reported record of a trombiculid mite from *Aplodontia*. The peculiar scutum of *T. aplodontiae* readily identifies the species.

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A NEW GENUS, *BORINQUOLAELAPS*, AND NEW SPECIES OF MITES FROM RATS IN PUERTO RICO

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The increasing importance of rickettsial diseases in the Americas makes necessary a more complete knowledge of the ectoparasites of rats. While rat fleas have been continuously and thoroughly studied but little attention has been paid to the mites. No species of mites have heretofore been reported from rats in Puerto Rico, although very thorough rat flea surveys were made by Carrion (1927–1932). In the present paper five known species are recorded and seven more are described as new. All the material on which this paper is based is in the collections of the Department of Medical Zoology, School of Tropical Medicine, San Juan, Puerto Rico.

The field work was done at Camp O'Reilly, Gurabo, in the interior of the Island (April 17 through October 9, 1945) and at San Juan on the north coast (January 17 through April 30, 1946). At Gurabo 238 rats and mice were collected by means of guillotine traps which killed the animals. The host species collected in order of abundance were: *Rattus norvegicus, Mus m. musculus,* and *Rattus rattus* (subspecies were not separated). At San Juan traps were used which took the animals alive and here 104 rats were collected—*Rattus norvegicus* (82), *Rattus rattus* (22)—and three mice.

The question whether the new species described below are normal parasites of the rat or only accidentally upon them cannot be decided at this time. In the debris associated with rats occur representatives of many different insect orders—ORTHOPTERA, HYMENOPTERA, DERMAPTERA, CORRODENTIA, DIPTERA, etc.—and obviously non-parasitic mites, as TYROGLYPHIDAE, TYDEIDAE, CHELYTIDAE, etc. In this connection, however, it should be pointed out that Hill and Gordon (1945) have shown that obscure mites associated with rats may on occasion become annoying to man.

The identification of parasitic mites is extremely difficult at the present time because of the uncertainty of generic delineation both from the nomenclatorial and zoological points of view. Generic and specific characters have not been worked out and a state of chaos exists which will doubtlessly not be clarified for some time to come. Although the writer has been at pains to secure and follow the opinion of contemporary workers, they have not been able to suggest better arrangements than those presented in this paper. One thing is certain, that names should be given to the species involved so that a starting point may be made. Jellison (1945), by describing a species even though it was not possible to ascertain its proper generic affinities, made a good start in the right direction. Names must be given to the species of mites parasitic on rats not so much for taxonomic reasons, as for experimental ones. For this is the first step in attempting to ascertain their significance in the transmission of disease.

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Family Liponyssidae

Bdellonyssus bacoti (Hirst)

1913. *Leiognathus bacoti,* Hirst, Bul. Ent. Res. **4:** 122.
1914. *Leiognathus bacoti,* Hirst, Ibid. **5:** 225.
1922. *Liponyssus bacoti,* Ewing, U. S. Natl. Mus. Proc. **42:** 18.
1923. *Liponyssus bacoti,* Bishopp, U. S. Dept. Agr. Circ. 294.
1923. *Liponyssus bacoti,* Cory, Jour. Econ. Ent. **16:** 226.
1929. *Liponyssus bacoti,* Ewing, A Manual of External Parasites, p. 15.
1929. *Liponyssus bacoti,* Hasseltine, U. S. Pub. Health Serv. Rpts. **44:** 587.
1931. *Liponyssus bacoti,* Shelmire and Dove, Jour. Amer. Med. Assoc. **96:** 579.
1931. *Liponyssus bacoti,* Dove and Shelmire, Ibid. **97:** 1506.
1931. *Liponyssus bacoti,* Fox, U. S. Pub. Health Rpts. **46:** 575.
1932. *Liponyssus bacoti,* Dove and Shelmire, Jour. Parasitol. **18:** 159.
1932. *Liponyssus bacoti,* Ewing, Wash. Ent. Soc. Proc. **34:** 14.
1940. *Liponyssus bacoti,* Riley, Jour. Parasitol. **26:** 433.
1942. *Bdellonyssus bacoti,* Fonseca, Butantan Inst. Mem. **16:** 153.
1945. *Liponyssus bacoti,* Rumreich and Wynn, U. S. Pub. Health Serv. **60:** 893.

Remarks.—This widely distributed species has frequently been reported as a pest of man in various parts of the world. It is also implicated in the transmission of endemic typhus fever. In the United States it is known from California, District of Columbia, Florida, Georgia, Iowa, Louisiana, Maryland, Massachusetts, Mississippi, Missouri, Minnesota, New York, North Carolina, Ohio, Tennessee, Texas, Virginia and Washington. In Puerto Rico it was collected both at Gurabo and at San Juan. In all 22 specimens were obtained, for the most part immature, from *Rattus norvegicus* and *R. rattus.*

Family Cheyletidae

Myobia musculi (Schrank)

1781. *Pediculus muris musculi,* Schrank, Enumeratio Insect. Aust. Indig., p. 501.
1896. *Myobia musculi,* Osborn, U. S. Dept. Agr. Div. Ent. Bul. No. 5, p. 263.
1904. *Myobia musculi,* Banks, U. S. Natl. Mus. Proc. **28:** 20.
1934. *Myobia musculi,* Radford, North Western Nat., December, p. 40.
1938. *Myobia musculi,* Ewing, Washington Ent. Soc. Proc. **40:** 194.

Remarks.—This species is very common in Europe. Ewing (1938) records it from Delaware, District of Columbia, Maryland and Virginia, and Osborn (1896) reported it from Iowa and Nebraska. In Puerto Rico it does not seem to be very abundant as only three females were taken at Gurabo and two larvae at San Juan from *Mus m. musculus.* The larvae were found imbedded in the ear in tick-like fashion.

Radfordia ensifera (Poppe)

1896. *Myobia ensifera,* Poppe, Zool. Anz. **19:** 341.
1914. *Myobia ensifera,* Hirst, Bull. Ent. Res. **5:** 124.
1934. *Myobia ensifera,* Radford, North Western Nat., December, p. 41.
1933. *Myobia ratti,* Skidmore, Jour. Parasitol. **19:** 172.
1934. *Myobia ratti,* Skidmore, Canad. Ent. **66:** 112.
1938. *Radfordia ensifera,* Ewing, Wash. Ent. Soc. Proc. **40:** 188.
1945. *Myobia ensifera,* Rumreich and Wynn, U. S. Pub. Health Serv. **60:** 893.

Remarks.—This common European species is known in the United States from Florida and Nebraska, and Skidmore (1934) reported it from Canada. Many specimens were taken from *Rattus norvegicus* at Gurabo, but none were taken in San Juan.

Family Laelaptidae

Echinolaelaps echidninus (Berlese)

1887. *Laelaps echidninus,* Berlese, Acari, Myriopoda et Scorpiones hucusque in Italia reperta. Fascl. XXXIX, No. 1.
1908. *Laelaps echidninus* Miller, U. S. Pub. Health Serv. Hyg. Lab. Bul. No. 46, p. 25.
1913. *Laelaps echidninus,* Hirst, Bul. Ent. Res. **4**: 123.
1914. *Laelaps echidninus,* Hirst, Ibid. **5**: 119; 229.
1925. *Laelaps echidninus,* Fox and Sullivan, U. S. Pub. Health Serv. Rpts. **40**: 1925.
1929. *Echinolaelaps echidninus,* Ewing, A manual of Ectoparasites, p. 11.
1929. *Laelaps echidninus,* Hasseltine, U. S. Pub. Health Serv. Rpts. **44**: 587.
1931. *Laelaps echidninus,* Fox, Ibid. **46**: 575.
1935. *Laelaps echidninus,* Fonseca, Butantan Inst. Mem. **10**: 33.
1937. *Laelaps echidninus,* Womersley, Parasitology **29**: 530.
1945. *Echinolaelaps echidninus,* Rumreich and Wynn, U. S. Pub. Health Serv. Rpts. **60**: 893.

Remarks.—This is a common species on rats all over the world. In the United States it has been reported from Florida, Georgia, Massachusetts, New York, and Virginia. In Puerto Rico sixteen specimens were collected from *Rattus norvegicus* at Gurabo and 15 from the same host at San Juan; all were females.

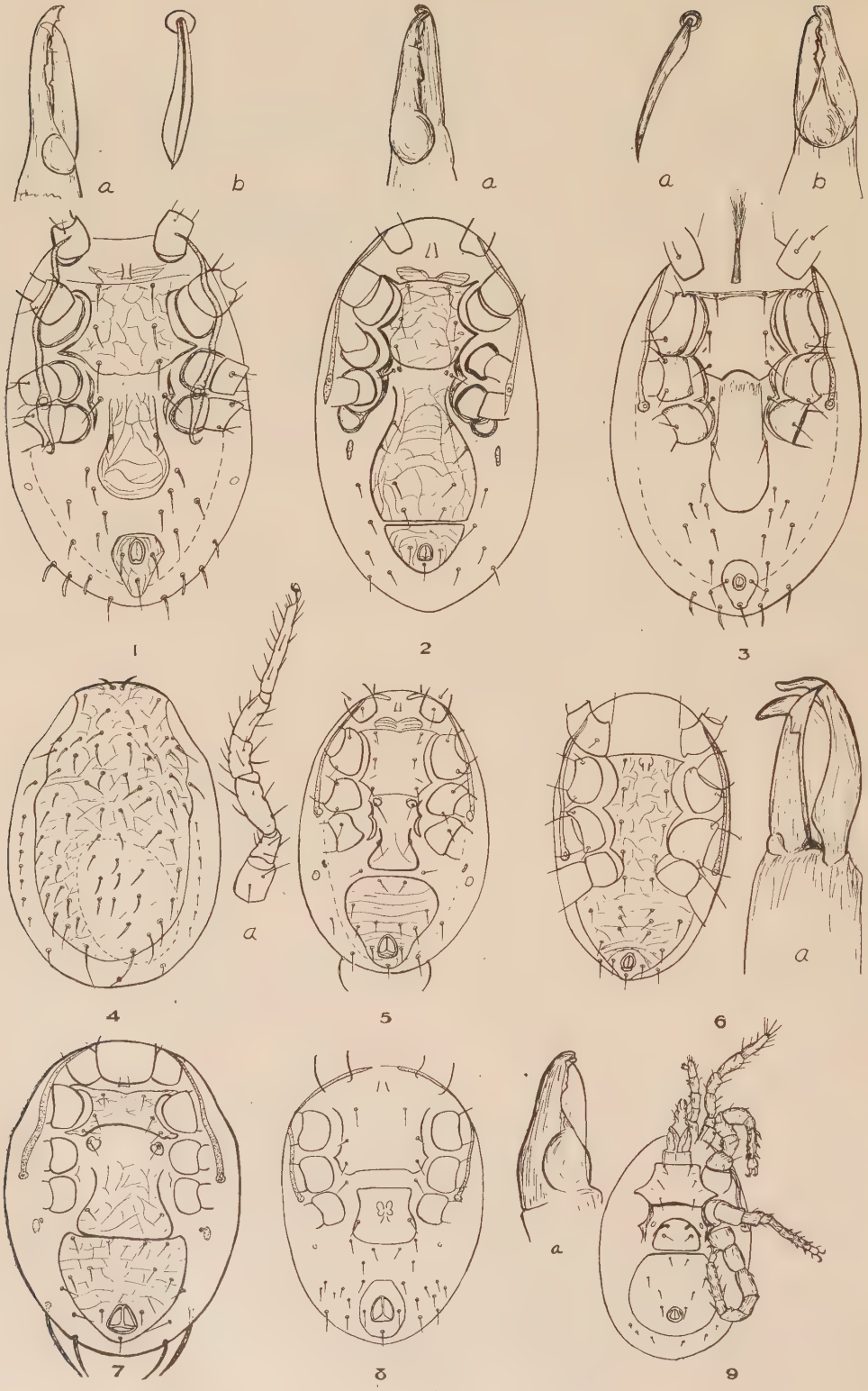
Laelaps nuttalli Hirst

1915. *Laelaps nuttalli,* Hirst, Bul. Ent. Res. **6**: 183.
1924. *Laelaps hawaiiensis,* Ewing, Bernice P. Bishop Mus. Bul. **14**: 7.
1925. *Laelaps hawaiiensis,* Fox and Sullivan, U. S. Pub. Health Serv. Rpts. **40**: 1925.
1929. *Laelaps hawaiiensis,* Hasseltine, Ibid. **44**: 587.
1935. *Laelaps nuttalli,* Fonseca, Butantan Inst. Mem. **10**: 33.
1937. *Laelaps nuttalli,* Womersley, Parasitology **29**: 530.
1945. *Laelaps hawaiiensis,* Rumreich and Wynn, U. S. Pub. Health Serv. Rpts. **60**: 893.

Remarks.—Ewing's *hawaiiensis* is a synonym of Hirst's *nuttalli.* Comparisons made at the writer's request with type material of *nuttalli* in the British Museum and material from the type host and locality of *hawaiiensis* in the U. S. National Museum (the type of *hawaiiensis* apparently being lost) substantiated this point of view. Specimens from Atlanta, Georgia, were found to be identical with those from Puerto Rico. Hirst in his original description reported the species from Ceylon, India, Sierra Leone and Dutch Guiana; and it seems to be cosmopolitan in distribution. In the United States it is known from Florida, Georgia, Louisiana, New York and Virginia, and Fonseca (1935) records it from Brazil. This species was the most common mite taken off rats in Puerto Rico, occurring in numbers on rats from both Gurabo and San Juan.

Ischnolaelaps alexandrini, n. sp.

Female: Body longer than broad, oval in shape, rather sparsely provided with setae; total length not including capitulum, 0.67 mm, width, 0.41 mm. Chelicerae (Fig. 3, *b*) large and prominent, toothed, fixed arm without a conspicuous process or seta. Hypostome plumose. Sternal plate (Fig. 3) broadest between coxae II and III, somewhat longer than broad, if the acuminated corners are not counted; with three pairs of moderately sized setae placed as usual and two pairs of normal pores. Metasternal setae level with the posterior borders of coxae III. Metasternal plates well sclerotized. Genito-ventral plate rounded posteriorly, posterior portion somewhat broader than the anterior portion; anterior border weakly sclerotized; provided with a single pair of setae located posterior to coxae IV. Approximately 12 setae are situated between the genito-ventral plate and the anal plate and in their near vicinity. Metapodal plates absent or very weakly sclerotized. Anal plate longer than broad, more or less triangular, with the anal pore closer to the anterior border than to the posterior one. Paired anal setae level with the middle of the anal pore; unpaired anal seta almost at the posterior edge of the anal plate and longer than the others. Posterior border of the ventrum with four prominent stout setae similar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to those found on the dorsal plate. Peritremes commencing between coxae III and IV and extending to or slightly before coxae I, tortuous and with uneven edges at least basally. Stigmal pore distant from the lateral border of the body. Coxae without prominent spine-like setae; chaetotaxy of the rest of the joints normal. Legs I and IV the longest. Dorsal plate not covering the entire dorsal surface, slightly sculptured, provided with many setae, each one prominent, long and stout (Fig. 3, *a*).

Male: Body longer than broad, oval, shorter than that of the female, 0.46 mm long and 0.29 mm wide. Movable arm of chelicerae (Fig. 6, *a*) double, the inner arm with a large prominent tooth. Ventral plates fused; five pairs of setae between the two rows of coxae, these are followed by ten more plus the usual three anal setae (Fig. 6). Peritremes less tortuous than in the female, reaching almost to the anterior borders of coxae I. Coxae without spine-like setae. Dorsal plate similar to that of the female and armed with the same type of setae, but more prominently sculptured.

Type Material: Female holotype and female paratype from *Rattus rattus* collected September 9, 1945; male allotype from *Rattus* species collected between April and October, 1945; at Camp O'Reilly, Gurabo, Puerto Rico.

Remarks.—This new species differs from other members of its genus particularly in that it does not have well-developed metapodal plates.

Cosmolaelaps gurabensis, n. sp.

Female: Body oval, richly provided with stout setae and ornately sculptured. Total length, exclusive of capitulum, 0.64 mm, width 0.42 mm. Chelicerae (Fig. 1, *a*) very large and powerful, toothed, fixed arm with a small seta. Hypostome plumose. Sternal plate (Fig. 1) prominently sculptured, preceded by a lightly sclerotized presternal plate, with three pairs of large setae and two pairs of slit-like pores normally placed. Metasternal setae level with the posterior borders of coxae III. Metasternal plates heavily sclerotized. Genito-ventral plate with one pair of setae, rounded posteriorly, sculptured, posterior portion half again as broad as anterior portion whose border is weakly defined. Approximately 19 setae situated between the genito-ventral plate and the anal plate and in their near vicinity. Anal plate longer than broad, more or less triangular, with the anal pore located in the anterior half. Paired anal setae level with the middle of the anal pore; unpaired seta not much longer than the paired ones, located well before the posterior edge of the anal plate. Posterior edge of the ventrum with about nine robust setae, similar in form to those of the dorsum. Peritremes wide, extending posteriorly well behind the stigmal pore, anteriorly reaching to beyond the posterior borders of coxae I. Coxae without spine-like setae. Dorsal plate not covering the entire dorsal surface, prominently sculptured, provided with stout setae of the form shown in Fig. 1, *b*.

Type Material: Female holotype from *Rattus* species or *Mus m. musculus* collected at Camp O'Reilly, Gurabo, Puerto Rico, during the period April 17 through October 9, 1945.

Remarks.—As other members of this genus, described by Berlese (1904), are myrmecophilous, the possibility that this species is a parasite or associate of ants rather than rats should not be excluded from consideration.

Mysolaelaps stigmatus, n. sp.

Female: Body oval, sparsely provided with setae, 0.67 mm long exclusive of capitulum, and 0.40 mm wide. Chelicerae (Fig. 2, *a*) large and toothed, fixed arm with a weak seta. Sternal

EXPLANATION OF PLATE

FIG. 1. *Cosmolaelaps gurabensis,* n. sp. Female, ventral view of body; *a,* chelicera; *b,* dorsal seta.

FIG. 2. *Mysolaelaps stigmatus,* n. sp. Female, ventral view of body; *a,* chelicera.

FIG. 3. *Ischnolaelaps alexandrini,* n. sp. Female, ventral view of body; *a,* dorsal seta; *b,* chelicera.

FIG. 4. *Borinquolaelaps dentatus,* n. sp. Female, dorsal view of body; *a,* Leg I.

FIG. 5. *Borinquolaelaps dentatus,* n. sp. Female, ventral view of body.

FIG. 6. *Ischnolaelaps alexandrini,* n. sp. Male, ventral view of body; *a,* chelicera.

FIG. 7. *Borinquolaelaps coronatus,* n. sp. Female, ventral view of body.

FIG. 8. *Borinquolaelaps mariposus,* n. sp. Female, ventral view of body.

FIG. 9. *Macrocheles fonsecai,* n. sp. Female, ventral view of body and legs of one side; *a,* chelicera.

plate (Fig. 2) preceded by a well-sclerotized presternal area, shield-shaped, sculptured, with three pairs of moderately sized setae and two pairs of inconspicuous pores normally placed. Metasternal setae weak, on level with posterior borders of coxae III, situated between a small plate and the heavily sclerotized metasternalia. Genito-ventral plate reaching to the anal plate, the posterior border truncate, bearing four pairs of setae. Opposite the second pair of setae on the genito-ventral plate are oblong metapodal plates about as long as the setae. Anal plate semi-spherical, the anal pore closer to the posterior margin of the anal plate than the anterior one; unpaired anal seta of the same size or weaker than the paired ones. In addition to the setae on the genito-ventral and anal plates there are about ten more on the ventrum. Coxae not armed with spine-like setae. Peritremes extending posteriorly behind the stigmal pore and reaching anteriorly to beyond the posterior borders of coxae I. The coxal pits of coxae IV bear each an unusual reduplication, reminiscent of the stigma of ticks in appearance. Dorsal plate covering the whole dorsal surface, densely provided with weak setae.

Type Material: Female holotype from *Rattus rattus* collected September 28, 1945, at Camp O'Reilly, Gurabo, Puerto Rico.

Remarks.—The stigma-like reduplication of the fourth coxal pits particularly distinguishes this species from others in its group.

Borinquolaelaps, new genus

Body oval. Chelicerae toothed, fixed arm with many even teeth, having a saw-like appearance. Sternal plate with three pairs of setae and two pairs of pores, varying in size and shape. Genito-ventral plate with but one pair of setae, short and truncate. Anal plate in some species broadly expanded, reaching to the genito-ventral plate, in other species not expanded and not reaching to that plate. Anal pore large, shield-shaped and prominent. Peritremes extending beyond the anterior margins of coxae I. Coxae without spine-like setae. Leg I with the chaetotaxy shown in Fig. 4, *a*. *Genotype: Borinquolaelaps dentatus,* n. sp.

Remarks.—In the character of the genito-ventral plate, this new genus is similar to *Lasioseius* Berlese, from which it differs in the shape of the anal pore. The three Puertorican species, described below, may be distinguished by the following key:

1. Anal plate not broadly expanded and not provided with setae in addition to the anal ones ..... *mariposus,* new species
   Anal plate broadly expanded and with setae in addition to the anal ones 2
2. Anal plate reaching the genito-ventral plate and contiguous with it. *coronatus,* new species
   Anal plate separated from the genito-ventral plate ..... *dentatus,* new species

Borinquolaelaps mariposus, n. sp.

Female: Broadly oval. Total length excluding capitulum, 0.46 mm, width, 0.38 mm. Chelicerae characteristic of the genus. Sternal plate (Fig. 8) weakly sclerotized, indistinct, corners not markedly acuminate. Genito-ventral plate poorly sclerotized, with a sculptured design in the form of a butterfly. Between the genito-ventral plate and the anal plate and in their near vicinity there are about 22 setae. Anal plate longer than wide, not broadly expanded. Anal pore characteristic, its setae all of about the same size. Peritreme basally about its width from the lateral border of the body, extending forward far beyond the anterior margin of coxae I. Mesopodal plates small and circular. Dorsal plate occupying most but not all of the dorsal surface, provided with many long straight setae.

Type Material: Female holotype from *Rattus* species or *Mus m. musculus* collected at Camp O'Reilly, Gurabo, Puerto Rico, during the period April through October, 1945. Female paratype from *Rattus norvegicus* collected at San Juan, Puerto Rico, January 22, 1946.

Borinquolaelaps coronatus, n. sp.

Female: Body oval, total length exclusive of capitulum, 0.42 mm, width, 0.32 mm. Chelicerae characteristic of the genus. Sternal plate (Fig. 7) very narrow, twice as wide as long, provided with three pairs of setae: the anterior pair of pores is apparent, but the posterior pair is not discernible. Sternal plate followed by a pair of post-sternal heart-shaped plates. Genito-ventral plate sculptured, truncate, contiguous with the anal plate posteriorly; provided with a single pair

of setae. Anal plate broad, wider than the genito-ventral plate, sculptured, provided with eight setae in addition to those of the anal pore. Anal pore characteristic, its setae all of about the same size. Laterad of the anal plate are two setae on each side. The posterior margin of the body is provided with two large ventral bristles. Peritremes removed from the lateral margins of the body by about twice their width, extending anteriorly to beyond coxae I. Two metapodal plates are present on each side, one larger than the other. Dorsal plate occupying the entire dorsal surface, weakly sclerotized, sparsely provided with very small setae, and possessing a pair of large curved bristles near the posterior margin.

Type Material: Female holotype from *Rattus norvegicus* at San Juan, Puerto Rico, collected January 31, 1946. Female paratype from *Rattus* species or *Mus m. musculus* at Camp O'Reilly, Gurabo, Puerto Rico, collected during the period April 17 through October 9, 1945.

Borinquolaelaps dentatus, n. sp.

Female: Body oval, total length, exclusive of capitulum, 0.50 mm, width, 0.37 mm. Chelicerae conspicuously dentated. Sternal plate (Fig. 5) preceded by sculptured pre-sternal plates, not itself greatly sculptured, bearing three pairs of setae and two pairs of pores normally placed, followed by a pair of small post-sternal plates, each provided with a seta. Genito-ventral plate not heavily sclerotized, very short, not extending far posterior to coxae IV, the posterior margin truncate, with a single pair of setae. Anal plate large, slightly broader than long, sculptured, provided with eight setae in addition to those of the anal pore. Anal pore characteristic with setae all of about the same size. A pair of setae is located between the genito-ventral and the anal plate; and near the posterior half of the anal plate, a pair of setae on each side. On the posterior border of the ventrum is a pair of long, curved bristles. There are two metapodal plates on each side, one smaller than the other. Peritremes narrow reaching beyond the anterior borders of coxae I, almost to the hypostome. Dorsal plate (Fig. 4) provided with robust scimitar-like setae, not occupying the whole dorsal surface.

Type Material: Female holotype from *Rattus* species or *Mus m. musculus* collected at Camp O'Reilly, Gurabo, Puerto Rico, during the period April 17 through October 9, 1945.

Family Macrochelidae

Macrocheles fonsecai, n. sp.

Female: Body longer than broad, oval in shape, sparsely provided with setae. Total length not including capitulum, 0.59 mm, width, 0.40 mm. Chelicerae large, armed with uneven teeth (Fig. 9, *a*). Sternal plate, unsculptured, with three pairs of weak setae and two pairs of pores, all normally placed. Genito-ventral plate weakly sclerotized, truncate posteriorly, bearing a single pair of weak setae, anteriorly with superimposed wing-like narrow plates. Anal plate large, broadly expanded reaching almost to the genito-ventral plate, provided with six setae in addition to the anal ones. Anal pore small, its setae small, the unpaired setae missing in the specimens available. Peritreme close to the lateral margin of the body, extending to coxae I. Coxae without spine-like setae. Tarsus I without claws; rest of the tarsi each with two claws and three or four pairs of spine-like setae. For further details see Fig. 9. Dorsal plate not quite occupying the whole dorsal surface, armed with a few weak setae.

Type Material: Female holotype and female paratype from *Rattus norvegicus* at San Juan, Puerto Rico, the former collected February 16, 1946, and the latter March 20, 1946.

ACKNOWLEDGMENTS

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THE RELATION BETWEEN PANTHOTHENIC ACID AND *PLASMODIUM GALLINACEUM* INFECTIONS IN THE CHICKEN AND THE ANTIMALARIAL ACTIVITY OF ANALOGUES OF PANTOTHENIC ACID[1]

STERLING BRACKETT, EMANUEL WALETZKY AND MARGARET BAKER

There is common agreement on the desirability of developing new chemotherapeutic agents on a less empirical basis than that of testing miscellaneous compounds, but such an attempt is handicapped by inadequate knowledge of the metabolism of pathogenic organisms. The possibilities of other approaches are illustrated by the results of McIlwain and Hawking (1943) who showed that hemolytic streptococci which are known to require pantothenic acid for growth are inhibited in development both *in vitro* and *in vivo* by pantoyltaurine, an analogue of pantothenic acid.

It was believed that if the nutritional requirements of *Plasmodium gallinaceum* were known, such information might be useful as a guide in the selection and synthesis of compounds to be tested as antimalarials. No satisfactory procedure for the *in vitro* cultivation of *P. gallinaceum* was available when this work began (Ball, et al, 1945) therefore, an investigation of its nutritional requirements was limited by the fact that *in vivo* cultivation must be used. A study of the pantothenic acid requirements of this parasite was chosen since the requirements of the host for this vitamin are well known (Jukes and McElroy, 1943) and since McIlwain's data showed that analogues of pantothenic acid may have chemotherapeutic activity.

PANTOTHENIC ACID REQUIREMENTS OF *Plasmodium gallinaceum*

It has been known for some time that dietary deficiencies of the host may increase the intensity of an infection, and this has been interpreted to be a result of an impaired defense mechanism (Culbertson, 1941). An indirect effect via the defense mechanism seems less likely in other recently reported experiments in which various infections have been reduced in intensity in deficient hosts (Becker, Manressa and Smith, 1943; Seeler and Ott, 1944). Such a reduction may reflect the inability of the parasites to satisfy completely their growth requirements in deficient hosts. Trager's (1943) suggestion that pantothenic acid deficiencies do not alter the course of a malaria infection was discounted since he was dealing with a different species of parasite (*P. lophuræ*) and since, as he points out, his results were open to question because of a concomitant partial biotin deficiency.

P. gallinaceum is a favorable subject for the study of pantothenic acid requirements since standardized infections of high intensity can be obtained (Survey of Antimalarials, 1941–1945) and the pantothenic acid requirements of its host, the chicken, are well known. For the studies reported here, White Leghorn chickens were rendered deficient in pantothenic acid by feeding them, from the time of hatching, a diet free of this vitamin but well supplied with all other known essential factors. The diet, devised and furnished by Dr. T. H. Jukes of Lederle Laboratories, was made up as follows:

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[1] From the Chemotherapy Division of the Research Laboratories of the American Cyanamid Company, Stamford, Connecticut.

		Parts
Mix I	Yellow corn meal (dry-heated at 120° C, 36 hours)	55
	Wheat middlings	25
	Commercial casein	12
	Ground limestone	2
	Steamed bone meal	1
	Iodized salt	1
	Whey adsorbate	2
	Choline chloride	0.15
	Manganese sulfate	0.05
	p-Amino benzoic acid	0.001
	Pyridoxine	0.0005
	Biotin concentrate	0.00002
Mix II	Mazola	270 g
	Vi-Delta concentrate (90,000 unit A; 11,250 unit B per g)	28 g
	Mixed tocopherols, 40% solution	2 g

Just before using, 2 g of mix II are added to 98 g mix I.

Control birds of the same age were fed the same basal ration supplemented with d-calcium pantothenate either as a single daily oral dose of 0.5 mg or as an ingredient of the diet at the rate of 3 to 4 mg per 100 g of diet. Both diets were fed throughout the entire experiment.

The birds on the deficient diet grew poorly and at 21 to 25 days of age were only half the control weight. From the second week on deaths were increasingly frequent and the classical skin lesions appeared in the corner of the eye. The birds were infected when marked differences appeared in the supplemented and the deficient groups (11 to 19 days of age).

Malarial infections were produced either by the intravenous injection of pooled sporozoites obtained by grinding infected mosquitoes, each bird receiving the sporozoites from about one mosquito; or by the injection of parasitized erythrocytes in the numbers indicated in Table 1. The course of the resulting infections was deter-

TABLE 1.—*Blood-induced infections of* Plasmodium gallinaceum *in pantothenic acid deficient chickens*

Exp. No.	Diet	Age in days at time of infection	Aver. wt. on 10th day of infection	% Erythrocytes parasitized on day indicated†					
				6	7	8	9	10	11
1 inoculum: 100,000 P.C.* per kilogram	Deficient	15	53 g	..	<0.1 (4)	< 0.1 (4)	< 0.1 (3)	< 0.1 (3)	0.1 (3)
	Supplemented	15	107 g	..	0.1 (7)	2.3 (7)	3.5 (7)	32 (7)	58 (7)
2 inoculum: 25,000 P.C. per bird	Deficient	11	70 g	0.1 (10)	0.3 (9)	1.3 (6)	1.0 (6)	2.1 (6)	3.0 (6)
	Supplemented	11	102 g	4.5 (19)	21 (19)	54 (18)	45 (15)	..	..

* P.C. = parasitized erythrocyte.
† Figures in ( ) indicate the number of birds on which the average is based.

mined by the examination of daily blood smears and the parasitemias are expressed as the number of parasites per 10,000 erythrocytes or as the percentage of parasitized erythrocytes. The results are summarized in Tables 1 and 2.

The trophozoite-induced infections (Table 1) are strikingly less severe in the pantothenate-deficient birds than in the supplemented control birds. The infections in the supplemented birds are typical ones for this species in normal hosts, and the

slight differences in experiments 1 and 2 are probably due to the differences in inoculum size.

There was also a reduction in the number of merozoites produced by segmenters in deficient hosts. The mean number of merozoites produced by 120 segmenters, 30 each from 4 different supplemented birds in Experiment 2 was 18.0 (range 15 to 30; standard error 0.25) while the mean for 164 segmenters (113 from three different days from 1 bird and 51 from two days from another) from deficient hosts was only 11.5 (range 6 to 22; standard error 0.24). Taliaferro and Taliaferro (1944) showed that fewer merozoites were produced by segmenters during the crisis of infections of *P. brazilianum* and Boyd and Allen (1934) found the same in infections by *P. cathemerium*. These authors suggested that this reduction in merozoite production might be a result of the defense mechanisms of the host. Since the reductions in merozoite numbers in our infections were seen at parasite levels far below those existing at the time of crisis in normal birds we feel that they are probably not a result of host immunity but more likely a direct result of the failure of the parasites to secure sufficient pantothenic acid for normal development.

This explanation is substantiated (see later) by the fact that analogues of pantothenic acid have the same effect on the erythrocytic phases of this infection as does the deficiency of pantothenic acid in the host. This latter evidence also indicates that the effect is specifically related to pantothenic acid deficiencies rather than a general effect due to inanition.

In contrast to the trophozoite-induced infections, the course of the sporozoite-induced infections in both deficient and supplemented groups is typical for this species, in our experience. The incubation period of the infection was not materially altered and the small differences in parasitemia, if significant, may be due to the effect of the pantothenate deficiency on the erythrocytic phase of these infections.

If we are correct in concluding that the reduction in the infections of *P. gallinaceum* in pantothenate-deficient hosts is due to a lowered blood concentration of this substance, then it is of interest to determine, if possible, what these concentrations are in normal and deficient chickens. A limited amount of data is available on the blood concentrations of pantothenate in our experiments. Blood samples taken on the 5th day of infection from 3 deficient and 3 supplemented chickens of Experiment 2 were analyzed for pantothenate by Mrs. D. Vroman of the Analytical Department of Lederle Laboratories using *Lactobacillus casei E* assay (Strong, et al, 1941). The average value for the deficient birds was 0.155 γ/ml (range 0.12 γ to 0.216 γ/ml) while the average value for the supplemented birds was 0.24 γ/ml (range 0.20 γ to 0.26 γ/ml). On the 13th day of the infection in this same experiment, blood specimens were taken from 3 additional deficient birds. The average pantothenate concentration was 0.18 γ/ml. Since these birds had survived longer than others in the group they may have had slightly higher pantothenate concentrations than the average for the group. It is interesting, but perhaps only fortuitous that the intensity of the infections in these three birds was directly correlated with the pantothenic acid contentration in the blood.

Other investigators with more material have found somewhat higher concentrations of pantothenate in the blood of normal chickens. Snell, et al (1940) reported the average concentration for 3 chickens to be 0.36 γ/ml (data converted from "mg units" as given in paper to γ per ml by using the factor 0.08 obtained from Snell

TABLE 2.—*Mosquito-induced infections of* Plasmodium gallinaceum *in pantothenic acid deficient chickens*

Exp.No.	Diet	Age in days at time of infection	Aver. wt. on 6th day of infection	Aver. No. parasites/10,000 rbc's on day indicated*				
				6	7	8	9	10
3	Deficient	15	57 g	5 (13)	480 (13)	1800 (13)	5100 (9)	7000 (7)
	Supplemented	15	115 g	300 (7)	2600 (7)	4700 (7)	7500 (1)	....
4	Deficient	19	62 g	....	12 (5)	750 (5)	4000 (5)	....
	Supplemented	19	118 g	....	118 (18)	800 (18)	8650 (18)	....

* Figures in ( ) indicate the number of birds on which the average is based.

by personal communication) while Trager (1943) reports the average for 20 chickens to be 0.308 γ/ml. Snell also found that the pantothenate concentrations in the blood of 3 chickens fed a deficient diet was 0.09 γ/ml. This suggests that the erythrocytic stages of *P. gallinaceum* may require concentrations of more than 0.1 to 0.15 γ pantothenate per ml of host blood for normal development.

THE RELATIVE ANTIMALARIAL ACTIVITY OF ANALOGUES OF PANTOTHENIC ACID

Since the above experiments suggested that the blood stages of *P. gallinaceum* require pantothenic acid in their metabolism, they encouraged the synthesis of a series of analogues of this vitamin by chemists in these Laboratories (Winterbottom, et al, 1946). Standardized infections of *P. gallinaceum,* both sporozoite- and blood-induced, which have been described in detail elsewhere (Survey of Antimalarials, 1941–1945), were used for determining the relative antimalarial activity of these analogues.

The compounds were incorporated in the diets[2] and the light schedule consisted of alternating three-hour periods of light and dark. Sporozoite-induced infections were produced by the intravenous injection of sporozoites from about one-tenth of a mosquito. The inoculum was prepared by grinding infected mosquitoes in serum, diluting to the desired extent, and chilling.

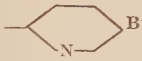
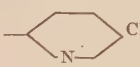
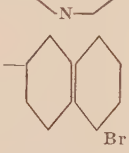
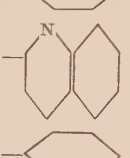
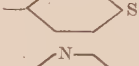
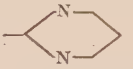
Trophozoite-induced infections were produced by the intravenous inoculation of 400 million or 200 thousand infected red blood cells per kilogram which respectively produced peak parasitemias 4 and 7 days later. The results from 4-day infections are more comparable to results obtained in routine antimalarial studies in other laboratories, as well as ours. The 7-day infections were used as a more sensitive test of the activity of slow acting compounds in which class it was thought the pantothenate analogues might fall. The results are expressed as a percentage derived by dividing the parasitemia of the test group by the parasitemia of the control group.

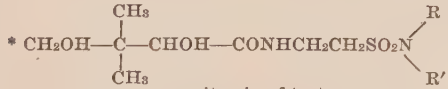
The 15 pantothenate analogues tested in blood-induced infections of 7 days' duration (Table 3) all showed some degree of activity and several of the compounds are more active than quinine in this test. Since no analytical methods were available it is not known whether the differences in relative activity are due to differences in inherent activity or in the pharmacological properties of the compounds. From

[2] Purina Brand Startena chick food used in all cases.

a practical standpoint, however, the more active of the series listed in Table 3 are markedly superior to pantoyltaurine which was inactive when administered intraperitoneally for three and one-half days at two-hour intervals with total daily doses of six grams per kilogram.

TABLE 3.—*Antimalarial activity of panthothenic analogues in blood-induced infections of* Plasmodium gallinaceum *of 7 days' duration*

No.	Compound* R	Compound* R′	No. of birds	Intake mg/kg/day	Activity†	Quinine equivalent
1			35	3.7 1.4 0.9	< 0.5 0.5 2.0	16
2			40	4.3 1.7 1.0	< 0.5 4.0 4.0	12
3			10	45 5	< 0.1 0.25	4
4			10	42 5.5	< 0.1 1.0	2
5			40	70 30 10	< 0.5 4.0 40	1
6			15	80 16	< 0.5 8.0	1
7			15	70 17	< 0.5 1.5	1
8			15	80 18	0.5 20	0.5
9			30	80 17	1 100	0.25
10		C_2H_5	30	75 15	< 0.5 40	1
11			25	135 70 33	< 0.5 2.0 43.0	0.5
12			25	680 140	1.0 14.0	0.25
13			10	175 20	< 0.1 10.0	0.5 (as dl mixture)
14			5	1150	< 1	active
15			5	2800	2	active



* $CH_2OH—C(CH_3)_2—CHOH—CONHCH_2CH_2SO_2N(R)(R')$

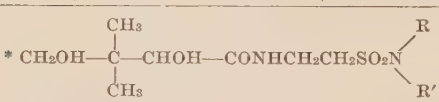
† Expressed as $\frac{\text{parasitemia of test}}{\text{parasitemia control}} \times 100$. Each figure is the average for a group of 5 birds.

Five of the more active members including compound #1 were tested in sporozoite-induced infections at doses of 400 mg/kg/day or higher but none showed the slightest degree of activity.

On the basis of the data given in Table 4, compound #1 (pantoyltauramido-4-chlorobenzene) is four times as active as quinine in this more severe test using blood-induced infections of 4 days' duration. The other compounds tested in 4-day blood-induced infections have quinine equivalents proportionately lower than those assigned them in Table 3. Attention is called to the fact that in both Tables 3 and 4 only representative data are given to illustrate the dosage-response relationships of the compounds, but the quinine equivalents are based on all of the data available. The difference in the quinine ratios in the 4- and the 7-day tests indicates that these

TABLE 4.—*Antimalarial activity of pantothenic analogues in blood-induced infections of* Plasmodium gallinaceum *of 4 days' duration*

No.	Compound* R	No. of birds	Intake mg/kg/day	Activity†	Quinine equivalent
1		80	6.5 3.5 2.0	0.5 2.0 11.0	4
3		30	17 8.3 2.2	< 0.5 14 28	2
2		30	16 9 4.4	5.0 22.0 45.0	2
16	— NO$_2$	15	120 32 8.2	< 0.5 < 0.5 15	1
4		10		< 0.5 20	0.5
17	—CH$_2$	15	35 9.5	0.5 40	0.5
13		10	78 41	10 10	0.3 (as dl mixture)



* $CH_2OH—\underset{\underset{CH_3}{|}}{\overset{\overset{CH_3}{|}}{C}}—CHOH—CONHCH_2CH_2SO_2N\begin{matrix}R\\R'\end{matrix}$

(see Table 3 for structures not given here.)

† $\frac{\text{parasitemia of test}}{\text{parasitemia of control}} \times 100$. Each figure is the average for a group of 5 birds.

compounds act slowly against *P. gallinaceum*. It may be of interest that the first compounds tested in this investigation were only active in the 7-day tests.

When the rate of action on erythrocytic parasites of the most active compound, #1, with 0.1% and 0.25% drug diets was directly observed by methods previously reported (Brackett, Waletzky and Baker, 1945) it was found to act much less rapidly than 0.2% quinine, but possibly somewhat more rapidly than 0.2% sulfadiazine. Thus, although many parasites showed morphological abnormalities 24 hours after introduction as small ring forms into drug-treated hosts, a minority developed into small segmenters with about ten segmenters each.

Pantoyltauramido-4-chlorobenzene (#1) and 2-pantoyltauramido-5-bromopyridine (#5) were both found to be active when administered orally as a single dose a day in a 7-day blood-induced infection. However, results similar to those with drug-diets were obtained only with two to five times as much compound.

Since the addition of pantothenate to the drug diet completely antagonizes the

action of two members of this series (Table 5) these compounds evidently interfere specifically with pantothenic acid metabolism. The relatively large amount of pantothenate in the diet required to antagonize the action of the analogues may be due to the fact that, because of rapid excretion or some such factor, it is difficult to raise appreciably the amount of pantothenate actually available to the parasites. White, et al (1946) have presented data from *in vitro* cultures of streptococci which indicate that one molecule of pantothenate can antagonize only 25 to 50 molecules of these analogues.

A limited amount of semi-chronic toxicity data is available from experiments using oral administration by the drug-diet[3] method. One-half of one per cent of #1 in the diet reduced food intakes markedly and resulted in the deaths of birds from the 12th day on. A diet containing 0.1 per cent of #1 (drug intakes of 120 mg/kg/day) resulted in only slightly reduced weight gains in a 10-day period, while weight gains were normal with a diet containing 0.025 per cent of drug (drug intakes

TABLE 5.—*Pantothenic acid antagonism of some panthothenate analogues in 7-day trophozoite-induced infections of* Plasmodium gallinaceum

Compound	No. of birds	Dosage	Ratio	Activity*	Degree of antogonism
		Test compound/Pantothenic acid	Test compound : Pantothenic acid		
		mg/kg/day			
1	5	9/72	1 : 8	100	Complete
	5	9/0		< 0.5	
	5	17/144	1 : 8	100	Complete
	5	17/68	1 : 4	57	Partial
	5	16/32	1 : 2	46	"
	5	15/15	1 : 1	8	"
	5	18/9	1 : 0.5	3	"
	5	18/0		< 0.5	
5	5	35/70	1 : 2	100	Complete
	5	30/0		4.0	

* Expressed as $\frac{\text{parasitemia of test}}{\text{parasitemia of control}} \times 100$. Each figure is the average for the group of 5 birds.

of 40 mg/kg/day). The apparent specific interference of these drugs with the pantothenic acid metabolism of the parasites suggests the possibility of their interfering with the pantothenic acid metabolism of the host. In another experiment one-half of one per cent of compound #1 in the diet resulted in the death of five out of five birds while birds given the same amount of drug in their diet but with the diet supplemented with 0.025 per cent calcium pantothenate (dextro rotary) gained weight normally and in all respects seemed to be unaffected by the drug. This suggests that at least part of the toxicity of these compounds may be due to a direct effect on the metabolism of pantothenic acid in the host. The wide range between the toxic and therapeutically effective doses in this particular host-parasite relationship is emphasized since the compounds would undoubtedly be safe drugs in any similar relationship.

In addition to the reduction in weight gains and deaths, compound #1 causes a change in the blood picture in treated chickens. There is a striking increase in the percentage of immature erythrocytes in the circulating blood which is related directly to the size of the dose of the compound and the length of treatment. After three days of treatment with compound #1 at concentrations of 0.1, 0.025, 0.006 and

[3] Purina Brand Startena chick food used in all cases.

0.00015 per cent in the diet the mean percentage of immature erythrocytes was respectively 15, 9, 2 and 2. The untreated controls showed 3 per cent of immature erthrocytes. There was no difference in the total erythrocyte count between the controls and the birds with the largest dose of drug. The percentage of immature erythrocytes in the group fed the 0.1 per cent diet of compound #1 was 0 before treatment and 4, 11 and 15 per cent, one, two and three days, respectively, after treatment was started. In another experiment with 10 days of treatment, doses of 0.1, 0.025 and 0.0067 in the diet resulted in immature erythrocyte counts of 26, 15 and 8 per cent, respectively. When this same drug was administered in the food to 5 adult rats at a dose level of approximately 0.25 gm/kg/day, the blood picture remained normal over a 30-day period (E. J. Robinson, personal communication).

Five other members of this series, namely pantoyltauramidobenzene (#2), 2-pantoyltauramido-5-bromobenzene (#5), pantoyltauramido-4-methoxybenzene (#4) pantoyltauramido-4-methyl benzene (#3) and pantoyltauramido-4-nitrobenzene (#16), were examined for toxicity by comparing the percentage of immature erythrocytes in each group of birds after 10 days of treatment with 0.1 per cent of the respective drugs with the percentage of immature erythrocytes in groups of birds treated with various concentrations of #1. These drugs altered the blood picture to a lesser degree than did pantoyltauramido-4-chlorobenzene (#1) and roughly in proportion to their antimalarial activity.

DISCUSSION

It is of considerable interest that the pantothenate analogues are active against the stages which are affected by a deficiency of pantothenate in the host and inactive against the stages which are not. This, combined with the fact that the activities of the drugs are antagonized by pantothenic acid strengthens the conclusion that the erythrocytic stages of *P. gallinaceum* require pantothenic acid.

The normal course of infections in deficient birds inoculated with sporozoites of *P. gallinaceum* suggests that the exoerythrocytic stages of this parasite either require far less pantothenic acid than the erythrocytic stages, or that the tissues in which they reside are more richly endowed with this substance than the erythrocytes. The latter seems possible since Snell, et al (1940) found that the pantothenate concentration in various tissues of both deficient and supplemented chickens may be 100 times that in the blood of these same animals. The concentration in the brain and liver (two favorable sites for exoerythrocytic stages (Huff and Coulston, 1944)) of deficient chicks may even be over 50 times that of the blood of normal chicks. Unfortunately no data are available on the pantothenate concentrations of the endothelial cells which are parasitized.

The range in antimalarial activity of Q0.25 to Q16 (Table 3) is in contrast to the range in antibacterial activity, both *in vitro* and *in vivo* for these same compounds (White, et al, 1946) which suggests that there is little or no difference in their inherent activity. Mrs. Edith Jackson of the Bacteriology Group in these Laboratories kindly determined the blood concentrations of compound #1 in chickens following single oral doses of 0.5 g/kg, using an assay procedure devised by her. The geometric means of the values for 6 chickens (250 g each in weight) at 1, 2, 4 and 6 hours after dosing were 8, 7, 4 and 2 mg/100 ml respectively with maximum concentrations as high as 16 mg/100 ml. These correspond closely with data ob-

tained from mice treated with the same dose of #1 and other members of the group. In contrast, compounds #5 and #9 at the same dosage never gave concentrations of more than 2 mg/100 ml. This difference in the absorption-excretion relationships may be the primary factor responsible for the range in antimalarial activity seen in our data rather than difference in inherent activity of the various analogues. This same factor may also account for the differences in toxicity seen in some of the compounds.

It is of course impossible to make any predictions concerning the application of a member of this series in the treatment of other malarial infections, particularly those of man or of other parasitic infections. We have found the pantothenate analogues to be ineffective against coccidiosis in the fowl and Hewitt at Lederle Laboratories (personal communication) was unable to show any activity against *P. lophurae* in the duck. In this connection Trager (1943) has found that an infection of *P. lophurae* was not affected in pantothenic-acid-deficient ducks. The critical factor is the pantothenic acid requirements of the particular species of parasite in question. Our data suggest that these compounds will, however, be ineffective against parasites even though they require pantothenic acid if they reside in hosts or tissues containing sufficient quantities of the vitamin to antagonize the inhibitory action of the drug. In addition, they will be impracticable in hosts particularly sensitive to the potential toxicity of the compounds. The overall value of these compounds in any particular infection can be learned only by trial. We feel that the wide spread between effective and toxic doses in gallinaceum malaria warrant the trial of the most active compounds in other protozoön infections of poultry.

We wish to acknowledge our indebtedness to Drs. R. O. Roblin, Jr., and J. P. English for their advice and suggestions concerning the chemical phases of the problem and particularly to Dr. Robert Winterbottom who made the compounds available to us.

SUMMARY

Blood- but not sporozoite-induced infections of *P. gallinaceum* are suppressed in pantothenic-acid-deficient chickens or in chickens treated orally with analogues of pantothenic acid. This suggests that the blood phases of this infection require pantothenic acid. The tissue phases may either not require the vitamin or find sufficient quantities in the tissues of chickens even when they are fed deficient diets or are treated with analogues of the vitamin.

The most active analogue tested to date, namely pantoyltauramido-4-chlorobenzene, is four times as active as quinine in a standard four-day trophozoite-induced infection and is sixteen times as active in a more sensitive test.

The suppressive action of the drugs is completely antagonized by the addition of pantothenic acid to the drug containing diet.

Toxicity was seen only at doses far above those required for a therapeutic effect.

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A PRACTICAL METHOD FOR MASS PRODUCTION AND TRANSFER OF *XENOPSYLLA CHEOPIS*

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In connection with endemic typhus experiments at the Eighth Service Command Laboratory, large numbers of *Xenopsylla cheopis* were required. This paper describes the method used for the mass production of this flea and their subsequent transfer.

Although the rat is the usual host of *X. cheopis,* a mouse was found to be much more desirable, because of the lesser quantity of urine excreted into the infested bedding.

A white mouse was placed in a standard glass animal jar at room temperature and infested with approximately forty fleas. Three inches of coarse wood shavings were placed in the bottom of the jar for bedding, leaving six inches from the top of the bedding to the lip of the jar, which is a greater distance than the usual vertical jump of *X. cheopis.* As an added precaution to prevent the escape of fleas, a screen lid covered with muslin was fitted over the jar. Several thousand fleas were reared in such cages; only a very few were observed on the lid, and none were known to have escaped.

After the mouse was infested with the fleas, the cage was left undisturbed for approximately one month. During this time the mouse was fed "rat checkers" and water-soaked carrots daily. No additional water supply for the mouse was required. The flea larvae that were produced fed on the mouse feces and other debris in the jar, and upon reaching maturity they pupated in the bedding. The jar should be kept fairly clean of uneaten portions of carrots and "rat checkers" since this material often encourages the growth of molds and scavenger mites.

When the new generation of fleas begin to emerge, the mouse is transferred to a fresh jar and a new colony started. The bedding from the old jar is transferred to a gallon glass jar with a standard screw-top cover. This transfer is made by pouring the bedding with the fleas, eggs, larvae and pupae, through a large glass funnel. An excellent funnel for this purpose may be made by cutting the bottom out of a gallon jar, and inverting it over the jar to receive the bedding. These jars are then held together by two screw-type jar lids that have been soldered together at their tops, after the center disk has been removed. The steep glass sides of the funnel make escape of the fleas impossible, and the large openings at each end of the jar enable the bedding to be poured freely and quickly.

When all the debris is in the new jar, a round piece of wire screen is placed over it and fastened in place by strips of adhesive tape (Fig. 1). The mesh of the screen is large enough for the fleas to crawl through freely, yet small enough to prevent the bedding from falling through when the jar is inverted.

As the fleas emerge from the bedding, they work their way to the surface and perch on the wire screen. When sufficient adults have emerged for use in the laboratory, a quart fruit jar is fastened to the mouth of the gallon jar by means of the

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two soldered screw-top covers (Fig. 1). The larger jar is then inverted and the fleas fall into the smaller jar. The jars must be perfectly clean and smooth to prevent fleas from gaining a foothold. *X. cheopis* is negative to gravity; as soon, therefore, as the large jar is inverted, some of the fleas on the screen make their way back into the bedding. When all of the fleas have left the screen either into the bedding or into the quart jar, the jars are unscrewed and the large jar set upright. After the fleas come back on the screen the operation can be repeated, more fleas then being transferred to the quart jar. The fleas may then be transferred from the quart jar

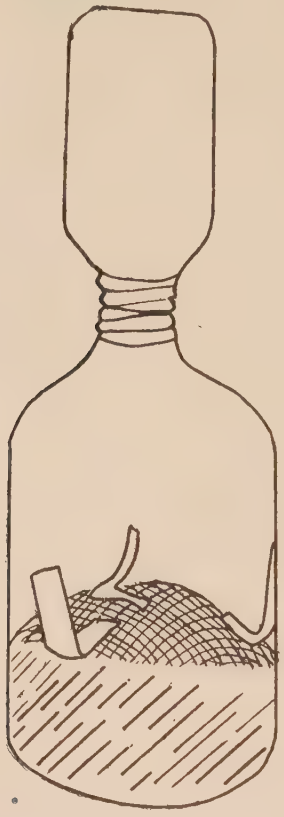

FIG. 1. Flea transfer jars.

to test tubes or other suitable containers through a glass funnel. Fleas will continue to emerge from the debris for approximately one month. Under optimum conditions more than 1,500 fleas have been obtained from one jar. During the hot summer months it is necessary to place the colony in an air-conditioned room at 70° F. for maximum production.

SUMMARY

A simple and efficient method of rearing and transferring *X. cheopis* in large numbers is described. A mouse infested with fleas is placed in an animal jar with wood shavings as bedding and left undisturbed for one month. The debris containing eggs, larvae, pupae and adults, is removed to a gallon jar. Adult fleas that emerge from this debris are transferred from this jar to smaller containers as needed. The entire operation is performed without the operator handling individual fleas.

A CYTOCHEMICAL STUDY OF THE "VOLUTIN GRANULES" IN PROTOZOA

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Purple staining granules are commonly found with the Romanowsky method in the cytoplasm of certain Sporozoa, Rhizopoda, Ciliophora and Mastigophora. They have been called "chromatoid grains" by Minchin (1909) and are generally referred to now as "volutin granules" on the assumption that they are constituted of the same substance which Meyer (1904) discovered in molds, yeast cells, and algae, and called "volutin." Meyer also concluded from certain microchemical reactions that volutin is related to the nucleic acids. Some protozoologists, considering the staining properties of the volutin granules, related the substance to chromatin and even considered it to be a precursor of that nuclear material. Others, on a less morphological basis, considered the volutin granules of protozoa as a cytoplasmic reserve substance. The latter opinion received much credit for it interpreted satisfactorily the fact that the granules appear, increase in size, or disappear in the life cycle of certain protozoa and in certain stages of trypanosomes in culture.

The recent progress in quantitative histochemical methods and qualitative cytochemical methods has made it possible to define more precisely the nature of cellular constituents.

Caspersson and Brandt (1941), using the ultraviolet absorption method, concluded that the volutin of the yeast cells was nucleic acid. Several authors, Brachet (1942) and others, used ribonucleases extensively to detect ribonucleic acid. Ribonuclease, a ferment extracted from the pancreas either by the method of Dubos and MacLeod (1937, 1938) or after successive precipitations by ammonium sulfate, specifically splits ribonucleic acid. When a cellular structure stains red with pyronine, or blue with toluidin blue, or purple with Romanowsky stains, and when it does not stain by the same methods after a preliminary digestion with ribonuclease, one can positively conclude that the staining of the structure was due to the presence of ribonucleic acid. The method is easily applicable to blood smears after Zenker's fixation (van den Berghe, 1942a). When very active, the ferment digests the stainable elements completely in less than an hour at 37° C. The digestion should be done at pH 6.8. Control slides in physiological saline must be run under the same conditions.

We have been able to demonstrate previously (van den Berghe, 1942b) that the "volutin granules" of the cytoplasm in trypanosomes (*T. evansi* and *T. gambiense*) disappear after the action of ribonuclease. By the use of a less active ferment and by varying the duration of the digestion, the granules are reduced gradually in size, suggesting clearly that the disappearance of the staining is related to a progressive destruction of ribonucleic acid.

Volutin granules in haemogregarines of the frog react in the same way.

We may therefore conclude that the so called volutin granules are the site of accumulated ribonucleic acid. They may be exclusively constituted by that chemical

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substance, but this cannot be ascertained by the method. At the present time we can only propose that the physical appearance and the staining properties of the granules are due to the presence of ribonucleic acid. This substance, which is found characteristically in the cytoplasm of all embryonic cells and of all very active adult cells, undoubtedly plays an important part at certain stages in the development of protozoa.

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A NEW LEECH, *PISCICOLA SALMOSITICA* N. SP. (PISCICOLIDAE), FROM STEELHEAD TROUT (*SALMO GAIRDNERI GAIRDNERI* RICHARDSON, 1838)

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The writer has received from ichthyologists various consignments of piscicolid leeches for identification and study. Early in 1942 a collection of twenty-six specimens, representing an undescribed species, was received through the courtesy of Dr. James E. Lynch, School of Fisheries, University of Washington.

One lot was collected by Mr. J. S. Dolley, at that time in the employ of the U. S. Fish and Wild Life Service, and supplied with the following information: "They were collected from sea-run steelhead trout (*Salmo gairdneri gairdneri*) which had been transferred from the Columbia River to Nason Creek, Chelan County, Washington. Presumably, the *Piscicola* infesting them came from Nason Creek, although they may have been brought from the Columbia River with the fish."

The following information accompanied the second lot: ". . . taken from the pond below the weir at the Soos Creek Hatchery near Auburn, King County, Washington. Soos Creek is a tributary of the Green River. The leeches were taken directly from the salmon, or collected from among the pebbles along the shores. These leeches congregate in the pond in large numbers during the spawning season of the salmon, most years. They are attracted not only by the salmon, but by the blood which is spilled in the water as the fish are killed and opened to obtain eggs."

Professor J. Percy Moore kindly made available a collection of thirty-four specimens, which he too had recognized as an undescribed species, received from Mr. Leo Shapovalov of Stanford University. They were taken from the same host, trapped at the Snow Mountain egg collecting station, Eel River, Mendocino County, California, February 1941.

The genus *Piscicola* Blainville, 1818, includes a total of nine species, all of which have been obtained from various species of fishes. Two species, *P. punctata* (Verrill, 1871) and *P. milneri* (Verrill, 1874), were originally described from the United States and *P. zebra* Moore, 1898, described from Nova Scotia. *Piscicola geometra* (Linnaeus, 1758), a species widely distributed throughout Europe and the British Isles, has been reported from Wisconsin and the District of Columbia. The writer has examined the above valid species. *Piscicola olivacea* Harding, 1920, and *P. cacca* Kaburaki, 1921, two species from India, are regarded as valid and provisionally belonging to the genus although the latter species, as a result of the absence of pulsating vesicles, fails to conform completely to the current generic diagnosis. If further investigation substantiates the absence of vesicles, an emendation of the generic concept will be necessary, if this species is to be retained in it. Otherwise, and wiser perhaps, subgenera must be established to accommodate it. For the purpose of the present study it is unnecessary to adopt either of the above alternatives

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and it is considered properly assigned *sensu lato*. Blanchard (1896) described *Piscicola elegans,* a fifth species, based on the external morphology of two specimens from China. Although his written description of the species may conform to the characters for the genus, the accompanying drawing appears to be incongruent with the generic concept. Two additional species, *Piscicola rectangulata* Levinsen, 1881, from Denmark and further reported from Alaska, and *P. platense* (= *P. platensis*) Cordero, 1933, from Uruguay and Argentina, although described as belonging to *Piscicola* clearly violate the generic concept. But until further study is made of these species it appears inadvisable to remove them from the genus. The descriptions of the nine species and specimens representing four of these species have been examined to determine whether the specimens under consideration may be assigned to one of them. But all the evidence is clearly negative and the material from the steelhead is here described as *Piscicola salmositica* n. sp.

Piscicola salmositica n. sp.

With the characteristics of the genus *Piscicola,* as emended by the writer (Meyer, 1940) in restricting the number of paired testes to six. Body width uniform; undivided into trachelosomal and urosomal regions. Ratio of over-all length: width :: 6 : 1. Two distinct pairs of pigmented eyes located in the posterior half of the anterior sucker. The eccentrically attached anterior sucker, measuring about 1.7 mm in diameter, concave ventrally, strongly discoidal, with the proboscis opening located in the posterior half. The broad, centrally attached posterior sucker, measuring about 2.8 mm, is strongly developed and distinguished by being slightly angular posteriorly. Eight or ten, or occasionally nine, strongly crescent-shaped pigmented spots, arranged diametrically to the longitudinal axis, around the postero-lateral margin of the sucker. Body and suckers may or may not be sprinkled with a more or less disorderly array of stellate flecks. Lateral pulsating vesicles, beginning on somite XIII, continue metamerically through XXIII. Anus opens between the third and fourth body rings anterior to the point of posterior sucker attachment. Gonopores, the anterior being distinguished by the disproportionately large atrium, are separated by two annuli. A distinct seminal vesicle lacking; anterior portions of vasa deferentia long and much convoluted before joining the very large bursa; paired ovisacs long, reaching to second pair of testes. Stomach possesses pronounced paired, lateral extensions between adjacent testes. Intestine begins in XIX and characterized by five paired digitate expansions before opening into the large rectum. Gastric caecum characterized by five, laterally orientated, ellipsoidal lacunae.

Holotype deposited U. S. National Museum Helminthological Collection, number 20,803. Paratypes in the collections of Professor J. Percy Moore, Professor Harley J. Van Cleave and the writer.

Both stained, in the manner described by the writer (1940: 335), and unstained specimens were studied for gross morphological details. Clove oil was used as a clearing agent. In addition to the study of *in toto* material, sagittal, frontal and transverse serial sections were necessary for the reconstruction of the finer internal morphological details. Sections were cut at 20 μ, stained with Delafield's hematoxylin and counter-stained with eosin. All figures were drawn with the aid of a projection microscope.

Although the means for the respective measurements of individuals of the Washington and the California collections are at a variance, the difference is insignificant and is regarded of no taxonomic consequence. Information concerning the manner of preservation is available for neither collection, but it is evident the specimens of each were subjected to different methods of treatment. The Washington material had been anesthetized; being well extended, straightened and somewhat flattened before the killing reagent was added. Only eight of the twenty-six specimens were unsuitable for measurement. Those from California were apparently placed di-

Size (mm) of *Piscicola salmositica* n. sp.

	Over-all length			Body breadth at widest place			Oral sucker diameter			Posterior sucker diameter		
	Range	Mean	Grand mean	Range	Mean	Grand mean	Range	Mean	Grand mean	Range	Mean	Grand mean
Washington specimens (eighteen)	10–31	20.7	19.7	1.5–6.0	3.5	3.3	1.0–2.0	1.3	1.7	1.5–4.0	2.3	2.8
California specimens (twelve)	14–23	18.2		2.5–4.0	3.0		2.0–2.5	2.2		3.0–4.0	3.5	

rectly into the killing solution without having been previously treated with an anesthetizing reagent. The result was that the specimens were curved ventrally and greatly contracted; only twelve of the thirty-four specimens being favorable for measurement.

The external morphology of leeches is subject to considerable variation, depending upon the method of killing employed. The temporary condition of the digestive system, materially affecting the size and shape of the animal, likewise introduces another variable. It is axiomatic then that size, *per se,* is of only minor taxonomic value. The true worth of such measurements assumes its greatest value when used comparatively and not as linear dimensions only.

In an earlier article (Meyer, 1940) the writer emphasized the importance of a knowledge of the internal morphology in the description and determination of valid species. Unfortunately such indispensable information has been all too absent in earlier descriptions, which has resulted in considerable taxonomic chaos within the group.

A more salient difference between the specimens from Washington and those from California was the presence or absence of stellate-shaped pigmented flecks on the body and extending onto the suckers, and the number of crescent-shaped spots on the posterior sucker.

Of the twenty-six Washington specimens only three were characterized by having numerous stellate flecks distributed over the body and suckers; while the remaining twenty-three were unflecked. Twenty-five specimens had eight pigmented, crescent-shaped spots on the posterior sucker; only one had nine and none were observed with ten such spots.

Among the thirty-four California specimens twenty-six were flecked, while only eight were unflecked. Only six specimens had eight pigmented spots on the posterior sucker, two had nine, leaving twenty-six with ten spots.

Such minor details as differences in size, the presence or absence of these body flecks and the number of pigmented spots on the posterior sucker are considered as nothing more than variations within the species. Since these two variable characteristics were uncorrelated among members of any one group or between the two groups and no significant internal morphological differences were observed in the leeches from the two localities they are considered together as one species. The size and shape of the body and suckers (Fig. 4) are consistent with other species of the genus.

The larger, somewhat variable, anterior pair of eyes, reaching to the mid-region of the anterior sucker, are crescent-shaped, with the convexities facing each other and placed at about a forty-five-degree angle anteriorly, with relation to the long body axis. The smaller dash-like posterior pair are placed in much the same position in relation to the posterior margin of the sucker.

Eight or ten, or occasionally nine, strongly crescent-shaped, pigmented spots are diametrically arranged, the convexities facing the longitudinal median axis, around the latero-posterior margin of the posterior sucker, with the largest and most characteristically shaped pair being posterior.

The body and suckers may or may not be covered with a more or less disorderly arrangement of thickly flecked, stellate spots. On the body these flecks are without pattern but show uniform spacing. On the anterior sucker, when present, these flecks are disposed chiefly around the margin, with a median line extending anteriorly

between the eyes. When present, on the posterior sucker, they likewise show no predominant uniform arrangement, except that nonflecked areas are observed between the pigment-crescents and the peripheral region of the sucker.

The pulsating vesicles, when in diastole, arch up in conspicuous little hemispheres of the skin and cover about four annuli. The location of these vesicles is in lateral apposition to the ganglion of the respective somites.

The tubular, muscular proboscis opens in the posterior half of the oral sucker and extends back to the ganglion of somite IX. At this point it receives the ducts of the numerous, conspicuous esophageal glands (measuring as much as 170 μ) located anterior and lateral to the point at which their ducts empty. The esophagus, into which the proboscis empties, joins the stomach at XII/XIII and is expanded. Characteristic of the stomach (Fig. 2) are the seven paired, lateral expansions, between which are located the six pair of testes. With the exception of the first, these expansions are digitate and show a tendency for undulations at the lateral margins. The size and the shape of these diverticula are somewhat variable, depending upon how recent the animal had fed, but even if they are not filled with food they tend to assume the same shape and degree of expansion as when the animal is gorged. At XIX, off the seventh stomach diverticulum, there arises a dorsal thick-walled intestine, which empties into the large rectum, and the stomach continues back to XXV as a thin-walled gastric caecum. Characteristic of this gastric caecum are the five ellipsoidal, laterally directed, lacunae located in apposition to the deep indentations separating the strongly bi- or tricrenulated, paired diverticula. Since each lacuna occurs in juxtaposition to a neural ganglion, it follows that each diverticulum of the caecum, with the exception of the last, occupies a part of two consecutive somites. The intestine possesses four large paired antero-lateral and a fifth smaller, laterally directed diverticula. The intestine empties into the large thin-walled rectum which in turn opens between the third and the fourth body rings anterior to the base of the posterior sucker.

The six pairs of testes (Fig. 3), as is characteristic for the genus, are placed intersegmentally between XII and XIX. Arising from each testis and continuing latero-anteriorly is a vas efferens which joints the lateral vas deferens from its respective side. Slightly posterior to the twelfth ganglion each vas deferens curves inward, twists beneath itself, before proceeding anteriorly. The vasa deferentia may lie either dorsal or lateral to the bursa. At about the level of the tenth ganglion each vas deferens turns and continues postero-median, running parallel to its own arm, for a short distance before looping again inward and continuing anteriorly. At the most anterior level, between ganglion nine and ten, each assumes several, somewhat variable sinuosities before continuing medially and posteriorly (ventral to the ascending arm), emptying into the unusually large and well-developed bursa. The large transverse atrium is located in the region of XI and XII. The female reproductive system consists of long (reaching ganglion fourteen and the second pair of testes), paired ovisacs which open in a small common ovipore, two annuli (280 μ) posterior to the male opening. The ventral copulatory area and specialized vector tissue described and figured by Brumpt (1901 : 18, 66) for *Piscicola geometra* was not observed to occur in *P. salmositica*. Whether or not the presence and possible disappearance of this area and accompanying tissue is correlated with periods of fecundation is unknown.

The nervous system consists of twenty-three ganglionic masses, representing thirty-four somites. The cephalic ganglionic mass, which encircles the proboscis sheath, represents six closely joined neuromeres and the posterior mass seven; leaving twenty-one free individual ganglia, metamerically disposed, between the two masses. In the mid-body region these ganglia are separated by about equal distances but toward both ends they are progressively crowded closer together and in the gonopore region (Fig. 1) their interdistances are inconstant.

When measured in a straight line from mid-ganglion to mid-ganglion the distance (in micra) between neuromere 8 (the second single, free ganglion) and 9 is 372; between 9 and 10, 335; between 10 and 11, 316; between 11 and 12 (characterized by great curvature), 930; between 12 and 13, and 13 and 14 each 538; between 14 and 15, and 15 and 16 each 614; between 16 and 17, 651 and continues constant posteriorly until entering the region of crowding. Although these measurements evidence inconstant spacing of ganglia they do not represent (in all cases) the true distances because of the curvature of the nerve cord connecting adjacent ganglia. The approximate actual distance, when measured (by the process of summation) on the curvature, between 11 and 12, where the curvature as well as the straight line distance is greatest, is 1395 micra. These measurements are based on a single representative specimen. This lack of uniformity between successive ganglia is caused by the disportionately large bursa, ventral to the nerve chain.

For this reason and the absence of sensillae, papillae or other similar metameric markings in this region, it appears inadvisable, if not impossible, to attempt to assign somite limits and/or fix the exact locations of the gonopores.

DISCUSSION

In regard to the relationship of *Piscicola salmositica* we find it occupies a position closest to *P. punctata* and *P. milneri,* although salient morphological differences readily distinguish it from either of the two. In *P. salmositica* the posterior sucker is centrally attached to the body while in both *P. punctata* and *P. milneri* it is eccentrically placed. Eight or ten crescent-shaped spots disposed around the margin of the posterior sucker serve as an aid in distinguishing the new species from *P. milneri,* which has ten or twelve punctiform spots. The posterior sucker of *P. punctata* is void of any such markings. The stellate body flecks on the new species when present, are not only smaller but without distinct pattern; distinct from *P. milneri.*

The reproductive and digestive systems, systems of real diagnostic importance, are distinct and quite striking in *P. salmositica.* The new species is readily distinguished from other known species of the genus by the tortuous convolutions of the vasa deferentia, prior to joining with the large bursa. The vasa deferentia of *P. geometra, P. punctata* and *P. milneri,* although characterized by sinuousities, differ in each species and in neither do they show the remarkable development present in *P. salmositica.*

The digestive system of *P. salmositica* is characterized by four large, paired antero-lateral and a fifth smaller, laterally directed intestinal diverticula. *P. geometra* possesses four large, but is without the fifth smaller pair; *P. punctata* possesses only three large pair before opening into the spacious, much diverticulated rectum. The shape of the five ellipsoidal, laterally directed, lacunae found in the gastric caecum of the new species is an unusual, if not unique, feature for the genus.

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Explanation of Plates

FIG. 1. Photomicrograph of a sagittal section through the gonopore region. Magnified 44 times.

FIG. 2. Dorsal view, showing in detail the digestive system and the body outline.

FIG. 3. Dorsal view of the reproductive system.

FIG. 4. Dorsal view, showing the external features. Sect. I. Anterior sucker together with adjacent annuli. Sect. II. Two complete somites in the region of the testes showing annuli. Sect. III. Posterior sucker together with adjacent annuli.

Abbreviations

Each scale line represents 2 mm.

A	anus	MG	male gonopore
AG	anterior ganglionic mass (1 through 6)	N	nerve cord
		O	ovary
B	bursa	P	proboscis
CD	caecal diverticulum	PG	posterior ganglionic mass (28 through 34)
CL	caecal lacuna		
E	esophagus	PV	pulsating vesicle
EG	esophageal gland or glands	R	rectum
FG	female gonopore	S	somite
G	neural ganglion	SD	stomach diverticulum
GC	gastric caecum	ST	stomach
I	intestine	T	testis
ID	intestinal diverticulum	VD	vas deferens
LM	longitudinal muscle	VE	vas efferens
M	mouth	VS	ventral sinus

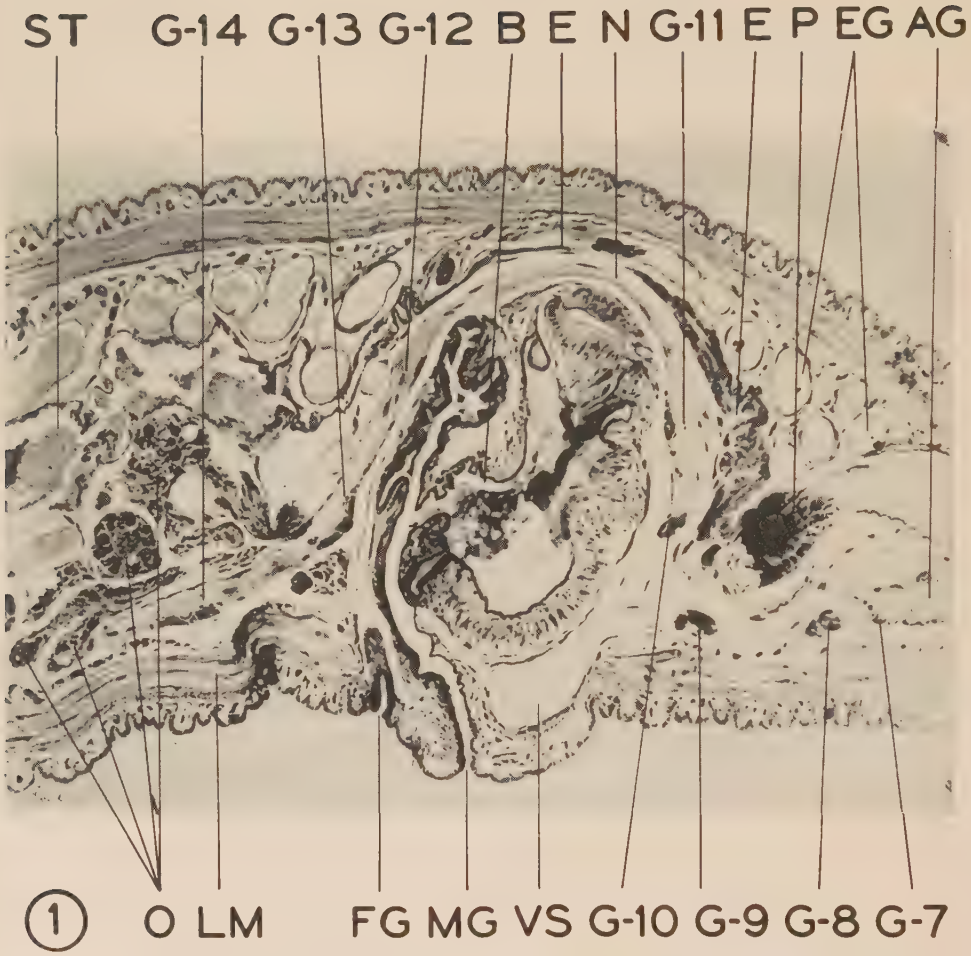

PLATE I

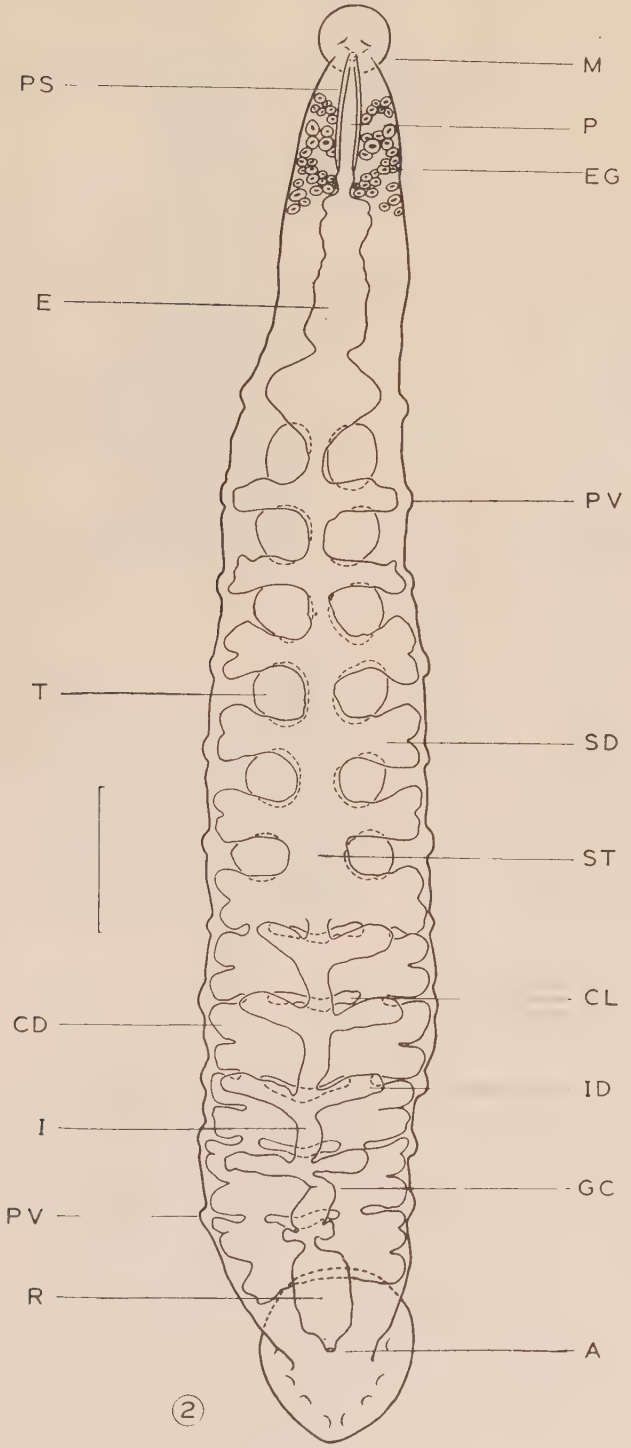

PLATE II

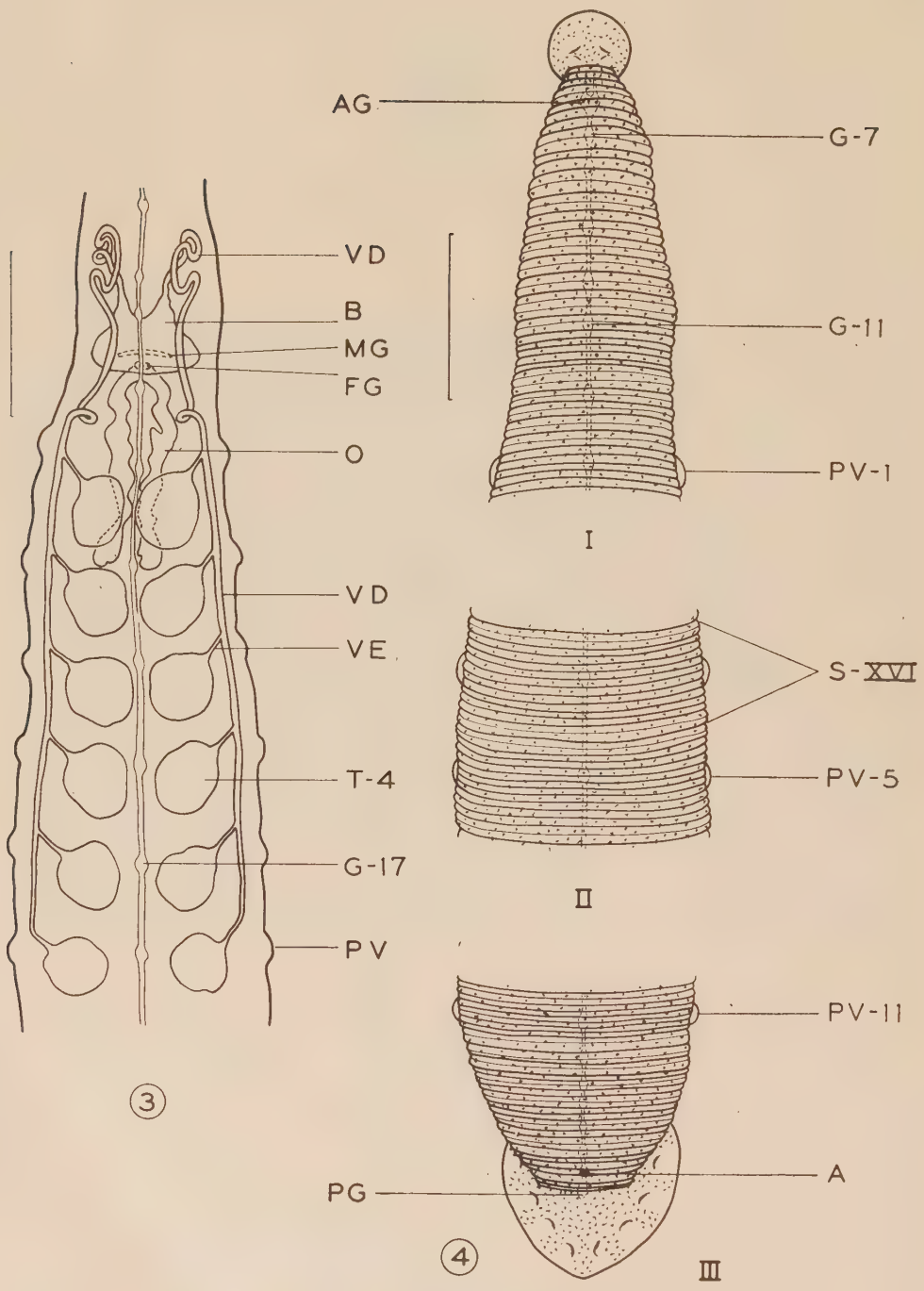
AG
G-7
G-11
PV-1
I
VD
B
MG
FG
O
VD
VE
T-4
G-17
PV
3
S-XVI
PV-5
II
PV-11
A
PG
4
III

PLATE III

A COMPARATIVE STUDY OF *HYMENOLEPIS* IN WHITE MICE AND GOLDEN HAMSTERS*

JOHN E. LARSH, JR.

Although golden hamsters, *Cricetus auratus,* are known to harbor *H. nana* (Stunkard, 1945), there appear to be no reports giving the percentage development of the parasite or other host-parasite relationships. However, other rodents, including rats, guinea pigs, deer mice, house mice, and brown laboratory mice have been compared with white mice in such respects (Shorb, 1933; Hunninen, 1935b; Larsh, 1944). In the present work with hamsters and white mice a comparison was made of the percentage development of adults, of the length of eleven-day worms, and of the extent of the prepatent and patent periods.

METHODS

The white mice were of the same strain used in all previous work and the hamsters were from a colony established with animals donated by a colleague, Dr. E. C. Pliske. The animals were raised *Hymenolepis*-free and when used were matched according to age and sex. Eggs for infections were isolated from fresh fecal pellets on the day of use. In all cases, counts were made of eleven-day adult worms. To facilitate length measurements, they were "relaxed" by placing them for a few minutes in tap water. Measurements were made of one-half the total number of worms in each animal and the average accepted. Prepatency, in all cases, was determined for individual animals. Examinations by the Willis method were made of samples of the total fecal output for a twelve-hour period daily, beginning the eighth day after infection. Patency, likewise, was recorded individually and was determined by continuing daily fecal examinations until seven consecutive samples were negative. The total absence of worms was then verified by autopsy.

Several experiments were carried out at intervals to compare, after similar infections, the percentage development of cysticercoids in 36 mice and a like number of hamsters, ranging from 1.0 to 2.3 months of age. The usual method (Hunninen, 1935a) was used in freeing the intestine of mucus preparatory to making cysticercoid counts. The method was, as always, satisfactory for mice, but in all hamsters this procedure removed with the mucus large numbers of villi. The results obtained were obviously inaccurate and cannot be presented. Therefore, a comparison of hamsters with other rodents as to the degree of susceptibility to cysticercoid development awaits a modification, or replacement, of this experimental method.

EXPERIMENTAL RESULTS AND DISCUSSION

Three experiments were performed at intervals to compare the percentage development and length of eleven-day worms in mice and hamsters (Table 1). In all cases, the hamsters showed a greater percentage development. In the first experiment, the average percentage development in the hamsters (7.4) was 2.2 times greater than the development in mice (3.4), and this ratio was about the same in

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TABLE 1.—*Showing the percentage development and average size of adult* H. nana *var.* fraterna *in white mice and golden hamsters*

Exper. no.	No. of animals	Age of animals (months)	Infecting egg dose per animal	Eleven-day adult worms		
				Average number	Average length (mm)	Percentage development
A. White Laboratory Mice						
1	5	1.0	3000	103.0	7.5	3.4
2	4	1.5	2000	85.5	8.0	4.3
3	5	1.5	2000	54.0	6.0	2.7
B. Golden Hamsters						
1	5	1.0	3000	220.4	17.5	7.4
2	4	1.5	2000	106.0	17.0	5.3
3	5	1.5	2000	114.8	15.8	5.7

the third experiment. The least difference was noted in the second experiment in which the average percentage for hamsters (5.3) was only 1.2 times greater than that for mice (4.3). A general comparison can be made of adult development in various hosts. Disregarding differences in age of animals and size of infecting egg doses used by various workers, the range of development has been found to be as follows for those rodents that appear to be the best hosts: white mice, 0.49–2.5 (Shorb, 1933), 1.0–8.0 (Hunninen, 1935b and c), 1.9–3.2 (Larsh, 1944); house mice, 1.5–2.5 (Larsh, 1944); brown laboratory mice, 1.0–1.9 (Larsh, 1944); and hamsters, 5.3–7.4 (Larsh, *vide supra*). Rats, deer mice, and guinea pigs, on the other hand, have shown striking resistance with always much less than one per cent development (Shorb, 1933; Hunninen, 1935b; Larsh, 1944). It appears, therefore, that of rodents tested, golden hamsters show the highest average percentage development of adult *Hymenolepis,* followed by white mice and house mice, and then brown laboratory mice. Rats, deer mice, and guinea pigs in that order, however, are much less suitable for this development.

Another point of interest in Table 1 is the length of eleven-day worms in the two hosts. In the one-month age group infected with 3000 eggs per animal, five mice had worms that averaged 7.5 mm in length, compared with an average length of 17.5 mm for those in hamsters. Thus the worms in hamsters were more than twice the length of those in mice, which was likewise found to be the case in the nine animals of each group 1.5 months old. Although measurements were not made, the worms in hamsters also were strikingly broader than those in mice.

Two experiments were carried out with animals 2.5 months old to determine the prepatent and patent periods of the worms in the two hosts. In the first experiment, nine animals of each group were infected with 1500 eggs per animal. The hamsters were first to show eggs, three being positive for the first time on the ninth day, two on the eleventh day, three on the thirteenth day, and one on the fourteenth day. Five white mice began passing eggs on the fourteenth day, two on the sixteenth day, one on the eighteenth day, and one on the twenty-third day. Similar results were obtained in the second experiment with twelve animals of each kind infected with the same number of eggs used in the first experiment. The worms in hamsters,

therefore, have a shorter prepatent period than those in mice, which was expected from the rate of growth in the two hosts. The patent period of the worms was likewise shorter in hamsters, ranging from 19 to 32 days, while in the white mice the range was from 27 to 62 days. It is interesting to note that worms in brown laboratory mice also show shorter prepatent and patent periods than those in white mice (Larsh, 1944). Perhaps in both cases this is a manifestation of an abnormal host relationship. It would be important, therefore, to know the extent of these periods in house mice, since they appear to be the most normal host for *Hymenolepis* (Larsh, 1944). In conclusion, it is clear that for use in experimental studies on a large scale using present methods, the white mouse still is the most suitable animal for many obvious reasons.

SUMMARY

The host relationships of *H. nana* var. *fraterna* were compared in white mice and golden hamsters. The hamsters showed a greater percentage development of adult worms, and their length at eleven days was much greater than those in mice. As was expected from this rate of growth, the prepatent period of the worms was shorter in hamsters. The patent period, likewise, was much shorter in hamsters.

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THE OCCURRENCE OF THE MITE *CHEYLETIELLA PARASITIVORAX* (MÉGNIN) IN NORTH AMERICA, WITH NOTES ON ITS SYNONYMY AND "PARASITIC" HABIT

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Since its discovery in France by Mégnin (1878), *Cheyletiella parasitivorax* Mégnin has been taken not infrequently from the fur of rabbits in Europe (early references in Oudemans, 1906; Pillars, 1925; Ford, 1944), and from the fur of angora rabbits from Auckland, New Zealand (Womersley, 1941). This mite has also been found rather uncommonly on the domestic cat in England (Hirst, 1917, 1922) and Denmark (Lomholt, 1918). Although *Cheyletiella parasitivorax* has been collected previously in North America from both rabbit and cat (*vide infra*), so far as could be found from a survey no clear record of the mite from this continent occurs in the literature.

During the interval from August 1943 to December 1944 the fur of more than 600 domestic cats from Maryland (143), Pennsylvania (239) and New Jersey (229) was examined for parasitic and pilicolous arthropods. Aside from the rather common occurrence of mange and six cases of infestation by the mallophagan *Felicola subrostrata* (Nitzsch), but one record of an infested cat proved especially noteworthy. The case in question was that of an adult male cat from Baltimore, Maryland, which was not mangy and appeared in good health. However its fur, especially between the shoulder blades, was literally teeming with *Cheyletiella parasitivorax* Mégnin in all stages of development. Identification of this striking mite is a simple matter; Banks' (1915) key, Oudemans' (1906) monograph and Hirst's (1917, 1922) excellent figure provide all that is needed for certain identification.

Recently, Vail and Augustson (1943) described a mite, *Ewingella americana* Vail and Augustson, which is said to differ from *Cheyletiella* by its broader palpi, type of tarsal claw, single dorsal plate, etc. Like *Cheyletiella parasitivorax* Mégnin, however, *Ewingella americana* occurs on the domestic rabbit. Examination of a paratype of *Ewingella americana* Vail and Augustson, kindly loaned for study by the Allan Hancock Foundation at Los Angeles, California, shows that the description of *Ewingella americana* is based upon specimens of *Cheyletiella parasitivorax* Mégnin. The palpi, the tarsal "claw," the dorsal plate, etc., of the paratype are all identical with those of *Cheyletiella parasitivorax* Mégnin, as are the other details of its morphology. Furthermore, although Vail and Augustson figured but two plumose setae in each dorsolateral angle of the "dorsal plate," there actually are three seta-pits on each side of the paratype examined—just as in *Cheyletiella parasitivorax* Mégnin. Apparently the most anterior plumose setae have been lost. In any event it is clear that *Ewingella americana* Vail and Augustson is a mere synonym of *Cheyletiella parasitivorax* Mégnin, and in this opinion I am joined by Dr. E. W. Baker of the Division of Insect Identification, U. S. Department of Agriculture (personal communication, Jan. 25, 1946).

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The infested Baltimore cat provides the second record of *Cheyletiella* from a cat and the ninth of eleven known occurrences from North America, although as stated earlier no specific record of this mite from North America occurs in the literature. Through the kindness of Drs. H. E. Ewing and E. W. Baker I am able to include here records from the files of the United States National Museum. *Cheyletiella parasitivorax* Mégnin has, then, been collected in North America as follows:

1919, Evanston, Ill.; from *Felis domesticus;* June 21 (T. V. Lacroix).
1930, Ancon, C. Z.; from domestic rabbit; Sept. 20 (L. H. Dunn).
1933, Ontario, Canada; from *Lepus americanus;* May 12 (D. A. MacLulich).
1938, Laurel, Md.; from rabbit; Dec. 8.
1939, Allegan, Mich.; from cottontail rabbit; Oct. 30 (A. O. Haugen).
1942, Albany, N. Y.; from rabbit; June 18 (Griffen).
1942, Savannah, Ga.; on flea on cottontail (C. N. Smith).
1942, Los Angeles, Calif.; as *Ewingella americana* Vail and Augustson; from domestic rabbit, Union Stock Yards; Dec. 17 (E. L. Vail).
1943, Baltimore, Md.; on *Felis domesticus;* Jan. 8 (K. W. Cooper).
1944, Mount Carmel, Conn.; on the flea *Odontopsyllis multispinosus;* April 28 (G. H. Plumb).
1944, Ithaca, N. Y.; from hair of cat; July 14 (G. J. Rubin).

Especially noteworthy in the above list are the two records of phoresy, a flea being the vehicle in both instances.

The role of *Cheyletiella parasitivorax* Mégnin in the fur of its host is an interesting problem. Neither the cat examined by me, nor Dr. Rubin's specimen, exhibited signs of damage by the mite. Nor were any other species of mite discovered on these cats, although in Hirst's (1917) case the cat was also infected with *Notoedrus.* According to Mégnin (1878) one finds *Cheyletiella parasitivorax* in the company of soft-bodied mites, such as listrophorids, upon which it feeds (thus representing Ewing's (1912) semi-parasitic stage in the evolution of a truly parasitic mite). Other workers have similarly found reason to believe *Cheyletiella parasitivorax* is of primary benefit to its mammalian hosts by feeding upon *Listrophorus, Psorergates, Myobia, Notoedrus,* etc., and this is the view generally expressed. It is quite possible in the first two cases mentioned above that the large *Cheyletiella* populations represented the concluding phases of an extermination of other cat parasites, or else the cats had recently acquired the populations by transfer from another animal—perhaps a rabbit. However, Pillars (1925) described a case of mange in domestic rabbits which he believed to be due to actual attacks of *Cheyletiella parasitivorax* upon its host. Pillars' evidence is not satisfactory, consisting merely in the demonstration of this mite under the heaped-up epidermal detritus of a hairless skin lesion, and the fact that brushing on alternate days cleared up the mange. Ford (1944) has recorded a similar case, but quite properly seems loath to attribute the atypical mange to *Cheyletiella.* Certainly the occurrence of *Cheyletiella* in advanced lesions is hardly to be counted evidence that *Cheyletiella* is the cause of these lesions. A similar criticism may be advanced to the conclusions of Vail and Augustson (1943) that *Ewingella* (= *Cheyletiella*) is a true mammalian ectoparasite. While it may eventually be demonstrated that *Cheyletiella parasitivorax* does attack its mammalian host, no compelling evidence that this is so has yet been brought forth.

In the light of the above, a special interest to parasitologists is awakened by

Pillars' (1925) rather under-cautious statement that two cases of mange in man were due to this mite, the infection being traced to cats. The source of this report is a brief note in Danish by Lomholt (1918) concerning a man and wife who developed red eruptions of the skin following the acquisition of a cat heavily infested with *Cheyletiella parasitivorax*. Although no mites were found on the patients, Lomholt was at first inclined to the belief that in fact mites from the cat had produced the lesions. But some five months later a similar case was discovered by his colleagues Jersild and Boas in which a similarly infested angora cat was involved. Since the mite was never found on the patients, Lomholt (1918) quite properly held it inadmissible to conclude *Cheyletiella parasitivorax* to be the cause of the itching lesion. In fact they held it more likely that cat or human fleas were the actual cause of the lesions. There is no evidence to date, therefore, which can be held to demonstrate that *Cheyletiella parasitivorax* Mégnin parasitically attacks its mammalian host, or that it can cause a mange in man.

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SUMMARY

(1) Records of *Cheyletiella parasitivorax* Mégnin from North American cats and rabbits are listed.

(2) *Ewingella americana* Vail and Augustson is reduced to synonymy with *Cheyletiella parasitivorax* Mégnin.

(3) *Cheyletiella parasitivorax,* as Mégnin (1878) first stated, is most probably a predator of soft-bodied mites parasitizing the mammalian host. There is as yet no conclusive evidence for the reports that *Cheyletiella parasitivorax* Mégnin attacks its mammalian hosts or that it is a cause of mange in rabbits and man.

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THE INTRACELLULAR BACTERIA OF THE COCKROACH IN RELATION TO SYMBIOSIS

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1. INTRODUCTION

The presence of intracellular bacilli in all species of cockroaches, so far investigated, has been known for many years. These bacteria are densely packed within the cytoplasm of certain specialized fat cells termed bacteriocytes. The microorganisms are transmitted from generation to generation through the ova.

To settle a disputed point, namely, whether the intracellular structures represented microorganisms or not, the writer (1930) cultivated the previously so-called "bacteroids" from two species of roaches by means of a special technique. It was found that the intracellular inhabitants of each host were represented by several types of a new species of diphtheroidal bacillus. Since 1930 the isolation and cultivation technique has been verified many times.

Are the intracellular diphtheroids symbiotic or benign parasitic microorganisms? The association is undoubtedly a biologically ancient one and there are good reasons for assuming that the relationship began as an invasion of roaches by vigorous parasites. Later, however, the invaders were partially controlled and rendered innocuous. Moreover, transmission from generation to generation through the ovum became established and this no doubt prevented the extinction of the parasites. Finally, it is conceivable that the association gradually assumed a form of symbiosis.

In a recent report Brues and Dunn (1945) give the effect of penicillin and certain sulfa drugs on the intracellular microorganisms of a species of Florida cockroach, *Blaberus cranifer*. They found that injections of sulfa drugs had no noticeable effect on the intracellular bacteria. Injections of high concentrations of penicillin, however, did affect the bacteria. Lower concentrations of penicillin greatly reduced the number of microorganisms, but after the insects were permitted to live normally for a time the bacteria gradually increased in numbers. When sufficient penicillin was administered to destroy the "bacteroids" or to reduce their numbers beyond a very low level, the roaches died after some days had elapsed. Brues and Dunn finally state: "We can not regard the present results as conclusive evidence that the bacteroids are necessary for the continued life of the cockroaches, but they make it appear very probable that such is the case and that they are symbiotic, and not parasitic, microorganisms."

2. EXPERIMENTAL

According to the writer's observations the diphtheroids within the fat body of normal males and females stain predominantly gram-positive with a few faintly staining gram-negative forms in the background. Cultural and other experience has proved that when the microorganisms react gram-negatively they are inactive or dead. Forms from ova usually react gram-positively, often showing cells in fission

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with pronounced banding and dark granulations. In preparing material for microscopical study of the condition of the intracellular inhabitants a word of caution may not be out of place. In staining one must be careful to check all solutions used in Gram's staining method with well-known gram-positive and gram-negative bacteria. All roaches must be examined for the diphtheroids immediately after the insects are killed, because after the death of the host the microorganisms change in their staining reactions. For instance, if the fat tissue of roaches is examined immediately, the majority of the diphtheroids are gram-positive. Twenty-four hours later many are gram-negative, after 48 hours all react gram-negatively, and after 72 hours all diphtheroids within the fat tissue are completely lysed. This occurs even if the material is stored in a refrigerator. Successful cultures can only be obtained when the microorganisms react gram-positively within tissues. It is always important to prepare and stain material from control roaches before interpreting the results of any experiment.

Untreated male and female roaches of approximately like age, in numbers equal to the treated individuals, accompanied all experiments as controls. These controls were all dissected and the state of the reproductive organs and the intracellular bacteria within them and within the fat tissue compared with the treated individuals.

The writer has been working for several years along lines similar to those of Brues and Dunn, using the roach *Periplaneta americana* as the experimental subject. When various doses of certain sulfa drugs were injected into the body cavity it was found that the intracellular bacteria were not noticeably affected. Several injections at time spaced intervals invariably killed the insects. Three different techniques were then developed which made it possible to obtain and keep under observation for considerable periods of time, roaches which were partially or completely free from intracellular bacteria.

a. *Prolonged Treatment with Sodium Sulfathiazole*

A prolonged drug treatment was used by incorporating sodium sulfathiazole into the drinking water of the roaches. Each insect to be tested was continuously supplied with 10 ml of distilled water containing 100 mg of sodium sulfathiazole. Prior experimentation showed that each mature roach drinks about 0.1 ml of water in 24 hours, so the daily dose of drug ingested would be about 1 mg. Each roach was also supplied with a small piece of lump sugar and a piece of dog biscuit. This biscuit, according to the analysis given by the manufacturers, is well-balanced nutritionally with respect to protein, fat, carbohydrate, vitamin, and inorganic constituents. Roaches thrive on this diet, and in the absence of the drug many were reared from eggs to adults and several lived up to two years.

In the first experiment four males were continuously treated with sulfathiazole. One of these received the drugged water for 150 days. At the end of that time the fat tissue was removed, triturated, stained and examined. The diphtheroids were plentiful, but all reacted gram-negatively. The testes and accessory glands were normal in appearance and motile spermatozoa were present in abundance. The second male was treated for 165 days and conditions were found similar to those of the first roach. The third roach, treated for 210 days, showed only a few isolated bacteria within the fat tissue bacteriocytes. These were gram-negative, swollen or abnormally elongated, granular and vacuolated. The testes were normal and con-

tained active spermatozoa. The last male in this series was treated for 406 days. At the end of that time no microorganisms were demonstrable in the fat tissue. The testes were normal as before. All four of these males acted in a normal manner and appeared well nourished judging from the accumulation of fat.

In another experiment five females were continuously treated with the sulfathiazole drinking water. In 60 days a slight effect on the fat cell bacteria of one female was noted. Another female was examined in 121 days, and no diphtheroids were found in any portion of the fat body. Ovaries were present but they were not fully developed. Two apparently mature ova were found but no bacteria were associated with them or with the follicles. Treatment for 255 days was practiced on the three remaining females which were immature. These matured within 60 days after the experiment was begun in so far as the acquisition of body size, wings, and external genitalia was concerned. On dissection not a trace of the ovaries could be found in any of these three roaches. No bacteria were discernible in the fat tissue of one roach. In another, two isolated gram-negative involution forms with irregular outlines were found. In the last female we encountered one partially intact fat tissue bacteriocyte with the remains of the diphtheroids exuding. These reacted gram-negatively and were in stages of pronounced disintegration. The above females acted in a normal manner and were well nourished.

b. *Treatment with Sodium and Calcium Penicillin*

Corynebacterium diphtheriæ and most strains of diphtheroidal bacilli are highly susceptible to penicillin. For this reason it was thought advisable to try the effect on the roach diphtheroids of both the sodium and calcium salts of this antibiotic. The salts were dissolved in sterile, distilled water[1] and a certain number of Oxford units injected into the body cavities of roaches.

Ten female roaches each received 2000 units of sodium penicillin: 1000 units twice at 24-hour intervals. Four individuals died following the second treatment. Six lived 20 days, when they were killed and examined. One possessed only one ovary and the fat cells showed a small number of gram-negative staining diphtheroids. The second female showed both ovaries only partially developed and the fat cells yielded a few gram-negative bacilli. The third individual showed well-developed ovaries with gram-negative and gram-positive bacilli within the ova and fat cells. No effect by the penicillin was noted in the fourth roach. The fifth had poorly developed ovaries with gram-negative staining microorganisms within the fat cells and three mature ova. The sixth female gave results comparable to the fourth individual.

Three males and six females received 3000 units of sodium penicillin on three successive days in three doses of 1000 units each. All but one female died within 5 days. The survivor lived 29 days when killed and examined. The ovaries were normal and the bacilli within the ova and fat cells appeared likewise.

Six females each received a total of 3000 units of sodium salt in two doses: 2000 units the first day and 1000 on the second. Of these roaches only two lived and were sacrificed on the 7th day. In one the fat tissue showed disintegrating diphtheroids and the ovaries were poorly developed. The second surviving female showed no change.

[1] Roaches will not tolerate the ordinary salt solutions commonly used.

Three males and three females received a total of 4000 units: 2000 units on each of two consecutive days. The males and one female died on the second day following the second dose. Two females lived for 24 days when sacrificed. A small number of gram-negative bacilli, apparently dead, were seen within a fat cell of one individual. None were found in the other. Ovaries were absent from both individuals.

Three females and three males each received a total of 6000 units of sodium penicillin in 2000-unit doses on three consecutive days. All died within five days without producing conclusive results.

Calcium penicillin was next used because in some cases this salt appears less toxic than the sodium form. Ten females and two males each received a total of 4000 units: 2000 units on the first day and 1000 on the second and third days, respectively. Two females died following the first dose and three females and one male died following the second dose. Five females and one male lived 83 days during which time they appeared well and active. On dissecting the females, it was found that the ovaries were entirely lacking, and microorganisms were absent from the fat cells. The male likewise showed no diphtheroids but the testes were normal with viable spermatozoa.

This experiment was repeated by using six females and four males. Two females and two males died following the second dose of the antibiotic. Four females and two males lived 66 days before they were sacrificed. During all of this time they appeared normal. The ovaries and diphtheroids within fat cells were absent from the four females. The two males were negative for diphtheroids but again the testes were well developed and contained viable spermatozoa.

It is seen from the above results that the mortality was also high when the calcium salt was used, but this appears less toxic than the sodium form and moreover gave more conclusive results.

c. *Treatment with a Raised Temperature*

The results with drugs seemed interesting enough to justify checking with a third technique. We therefore tried the effect of higher temperatures by means of an incubator.

The use of heat to free the host of invaders is not new. Cleveland (1924, 1925) with a number of techniques showed that termites could be freed of their intestinal protozoan fauna. Subjection to a higher temperature than the termites customarily encounter in nature was one of the successful methods used.

Kunkel (1936) showed that a number of plant viruses could be destroyed by subjecting the susceptible hosts to raised temperatures. For instance, by keeping potted trees, infected with peach yellows, in a hot room held at a temperature that varied from 34.4° to 36.3° C, a cure was obtained. The same worker (1941) also cured aster yellows in periwinkle plants by treatments in a hot room at 38° to 42° C. By employing a similar technique Braun (1943) killed the crown-gall bacterium, *Phytomonas tumefaciens,* the causative agent of a neoplastic condition known as crown-gall in plants.

Five of six roaches kept at 40° C died within three days. The survivor, an immature half-grown form, was treated for 12 days when it was examined. The bacteriocyte cells in the fat tissue were almost devoid of microorganisms. Only three or four gram-negative individuals were found. Subsequent experience showed

that the intracellular bacteria were not affected much by temperatures below 39° C. Temperatures above this point, however, with few exceptions always caused the death of all roaches used. Because of this, the temperature was lowered and maintained at 39° C.

Two males were held at 39° C for 28 days after which they were removed to room temperatures for 150 days. Dissection showed no diphtheroids within the fat tissue. The testes were normal and contained active spermatozoa. Twelve newly hatched nymphs from one egg capsule were placed at 39° C for 36 days. Only one individual survived this treatment. This survivor was then held at room temperatures and matured into a male in 450 days. He was dissected in 631 days. The testes were normal with active spermatozoa. The bacteriocyte cells of the fat tissue showed only a few abnormal microorganisms. These were gram-negative, small, very thin and generally atypical.

One female was held in the incubator at 39° C for 25 days. At the end of this time not a trace of the ovaries was visible and no bacilli were found within the fat tissue. Two females were heat-treated for 28 days. At the end of this period one individual revealed partially absorbed ovaries. A few ova were present but no bacteria were found associated with them or with the follicular membrane. The fat tissue also did not reveal any microorganisms. The other female showed no trace of ovaries nor any bacteria within the fat tissue.

Two female roaches were heat-treated as nymphs for 33 days. They then remained at room temperatures for 590 days when they matured. These insects appeared normal and active throughout. They were dissected 51 days after reaching maturity. The ovaries were absent and no microorganisms were found within the fat cells.

3. ANALYSIS OF DATA

Before analyzing the data (Table 1) it might be mentioned that only mature males and females were used unless otherwise noted in the text. It was necessary to employ irregular numbers of roaches because the material had to be used as it became available.

With the sulfathiazole treatment nine roaches were used and nine survived. In males the diphtheroids were adversely influenced in three and destroyed in the fat tissue of one. In females the treatment influenced the microorganisms in the fat tisue of three and in the ovary of one, and destroyed them within the fat tissue of two. The ovaries were retarded in one and absent in three.

TABLE 1.—*Effect of treatments on intracellular bacteria in* Periplaneta americana

Sex	Treatment	No. of roaches used	No. of survivors	No. of roaches in which: Bacteria in fat tissue were modified	Bacteria in fat tissue were absent	Bacteria in ovaries were modified	Ovaries were modified	Ovaries were absent
♂♂+♀♀	Na sulfathiazole	4	4	3	1	..	..	..
♂♂+♀♀	" "	5	5	3	2	1	1	3
♂♂+♀♀	Na penicillin	9	0	..	..	..	..	..
♂♂+♀♀	" "	28	11	6	1	..	4	2
♂♂+♀♀	Ca "	6	3	..	3	..	..	..
♂♂+♀♀	" "	16	9	..	9	..	..	9
♂♂+♀♀	High temperature	9*	4	2	2	..	..	..
♂♂+♀♀	" "	5	5	..	5	1	1	4
Total	♂♂*	28	11	5	6	..	..	..
"	♀♀	54	30	9	17	2	6	18

* Including one immature form.

With sodium penicillin nine males were used and none survived. Twenty-eight females were used and 11 survived. In these the diphtheroids were influenced adversely within the fat tissue of six, and were eliminated within the fat tissue of one. The ovaries were modified in four and absent in two.

With calcium penicillin six males were used and three survived. The bacteria were absent within the fat itssue of the three survivors. Sixteen females were used and nine lived. The diphtheroids were absent from the nine and the ovaries were also missing from all.

With the higher temperature treatment eight males and one immature form were employed of which four survived. The bacteria were influenced adversely within the fat cells of two and absent in two. Five females survived the treatment. The diphtheroids were absent within the fat tissue of all five. They were adversely influenced within the ovaries of one and the development of the ovaries was retarded. In four the ovaries were missing.

All of the results enumerated above have a bearing on the problem but perhaps the most significant facts are that the diphtheroids were completely eliminated within six males and 17 females and that the ovaries were missing within 18 females. When this is compared with the same number of adult control insects of like age with microorganisms within the fat tissue and fully developed ova with their complement of microorganisms the results appear quite striking.

It will be noted from the data that except for the prolonged sulfathiazole treatment the mortality was high in all experiments. It is felt that this was not due to the influence of treatments on the diphtheroids, which in turn influenced the insects, but to a direct toxic effect of the drugs on the roaches, and in the case of the higher temperature to an interference with normal physiological functions, at least in males. Those males and females in which the microorganisms were eliminated, and females with suppressed or absorbed ovaries, acted in a perfectly normal manner, ate well and were well nourished. They were normal except that the capacity of the females for reproduction was entirely suppressed. In other words, given the proper diet, the roaches without diphtheroids did not appear to suffer in health. What would happen in nature to roaches without their microorganisms and without proper dietary supervision is another matter.

Periplaneta americana from the time of hatching normally reaches maturity in from 250 to 300 days. The abnormal prolongation of the juvenile period observed in some of the treated insects is probably connected with the condition of the intracellular bacteria and cannot be attributed solely to the suppression of the female reproductive organs because in all insects investigated to date these organs do not appear to exert any influence on the secondary sexual characters.

4. CONCLUSIONS

From the data presented it appears that the intracellular diphtheroids found within *Periplaneta americana* males and females can be adversely affected or destroyed by either sulfathiazole, sodium and calcium penicillin, or by maintaining the insects for a prolonged period at a temperature of 39° C. The roaches that survived treatment did not show visible ill effects although the intracellular bacteria were adversely affected or destroyed. The impression is gained that treatment prolongs the juvenile period. The treatment which eliminated the intracellular microorgan-

isms prevented the development or caused the regression of the female sex glands. The male sex glands were not influenced by the treatments. The writer feels justified, therefore, in concluding that the diphtheroids are in symbiotic relationship with their female hosts and are closely connected biologically with the development of the female sex glands, perhaps furnishing these with some constituent or constituents lacking in the diet. When one remembers that the bacteria are transmitted solely through the ovum of the roach this probable relation between the female reproductive organs and the microorganisms does not appear fantastic. The writer does not presume that the relationship found in the females represents the only one. He was, moreover, not able to find any significant proof for symbiosis in the males.

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NECATOR AMERICANUS AND *ANCYLOSTOMA DUODENALE* IN GUAM, LEYTE, AND OKINAWA, WITH A NOTE ON HOOKWORM EGG SIZES

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During the course of parasitological studies of natives and service personnel on Guam, M. I., in 1945, some hookworm counts after treatment were included to determine the species involved. In addition, worms from necropsies of natives of Guam and Okinawa became available for examination. While a more definitive statement would need to be based on a larger number than the 4056 hookworms reported upon, the consistency of the findings permits a provisional species index for Guam, Leyte and Okinawa. Advantage was also taken of the presence of pure and mixed infections of *Necator americanus* and *Ancylostoma duodenale* to test whether egg measurements at fecal diagnosis could be used to reliably indicate the presence of a particular species.

Some of the hookworms here classified were collected following treatment of Guamanians at the United States Naval Military Government Hospital No. 203 (Agana), and of service men in the wards of the Naval Medical Research Unit No. 2; others were obtained at necropsies performed on natives of Guam at the Agana Hospital by Commander H. M. Zimmerman, MC(S), USNR. Those from necropsies of Okinawans were examined through the cooperation of Commanders E. L. Benjamin, MC(S), USNR, and Zimmerman, and Lieutenant Commanders M. B. Franks, MC(S), USNR and Harold Fink, MC, USNR. Acknowledgment is made to these officers, as well as to the physicians and nurses of the pediatrics ward of the Agana Hospital, and to the medical officers and pharmacists' mates of NAMRU No. 2, who made the post-treatment collections effective.

Identification of all hookworms was made by the writer. It will be noted that no specimens of *A. braziliense* were encountered, although there are occasional references to it as a human parasite in this part of the world. Hookworms from Guamanian dogs all proved to be *A. caninum*.

Ancylostoma duodenale AND *Necator americanus* RATIOS FOUND

Four distinct groups of persons were sampled as regards the hookworm species with which they were burdened: natives of Guam; garrison force personnel on Guam; marines who had been in the Leyte operation and then returned to Guam; and natives of Okinawa. Data from them are summarized in Table 1.

Natives of Guam. Ten Guamanians, 7 of them pediatric cases, were hookworm counted after treatment and additional worms were obtained from 11 necropsies. Various parts of the Island were represented geographically. Thirteen or 62 per cent of the individuals had *Ancylostoma duodenale* exclusively, and 76 per cent of the 1708 worms were of this species. In one instance 337 *A. duodenale* in pure infection were recovered from the 24-hour post-treatment stools of a child 16 months old from the town of Merizo.

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While this seems to be the initial report of the predominance of *Ancylostoma duodenale* on Guam, its presence on the Island was noted first by Kerr (1911). Stiles (1906), following the receipt of specimens collected from a native child by Assistant-Surgeon O. J. Mink, USN, recorded *Necator americanus* on Guam as one of the earliest demonstrations of endemic infection with this species outside the borders of the U. S. A. References to the hookworm species identified on other Micronesian islands have not been found. In regard to South Pacific islands lying one to four thousand miles south to southeast of Guam, Lambert (1928) in a synoptic article noted that from Papua and New Guinea, among 23,487 hookworms classified as to species, there were only 40 ancylostomes, and stated: "In pure native stocks only necators are found except where there has been close association with Chinese or North Indians. . . . Only pure necator infections have been shown from the Solomon Islands, Ellice Islands, Tonga, the Cook Islands, and the New Hebrides. Fijians who have not come in contact with Indian immigrants, harbor only necator infections." However, Liebow and Hannum

TABLE 1.—*Species determinations of hookworms secured after treatment of service personnel, and of natives of Guam and Okinawa; also of worms from necropsies of natives*

Source	Individuals with			Total hookworms	*Necator americanus*	Highest worm counts
	Necator only	*Ancylostoma* only	Over 100 hookworms			
21 natives, Guam	None	62%	24%	1,708	24%	337 314
14 service men, Guam	64%	36%	7%	374	85%	108 71
23 Leyte-returned marines	13%	26%	22%	1,570	82%	388 371
42 natives, Okinawa	57%	5%	None	404	70%	79 46

(1945) reported recovery of 886 hookworms, exclusively *A. duodenale,* from 10 soldiers who had been in campaigns in Guadalcanal and New Georgia (Solomon Islands).

The finding of a high proportion of *Ancylostoma* instead of *Necator* in native Guamanians was unexpected, and it may not be the rule for Micronesia. Obviously difficult now is the decision as to whether *Ancylostoma* came with the original Chamorro stock from Indonesia or was brought in later by the Spanish conquerors. In favor of the latter explanation is the catastrophe that overtook the indigenous Chamorros at the hands of the Spanish in the last quarter of the 17th century, when a protracted 21-year war of extermination against them by the Spaniards is said to have reduced their numbers from more than 50,000 to a few hundreds. As the survivors were mostly women and children who were taken over by the Spanish soldiery, there ensued a period when *A. duodenale,* the species endemic in Spain, could readily have been established and disseminated in this Spanish possession.

Garrison Forces on Guam. Fourteen garrison force personnel (i.e., men who came to Guam after the military re-occupation in July-August, 1944) were worm counted after treatment, with a finding of 85 per cent *Necator americanus* among 374 hookworms. From a study of the medical and service records of these men it was apparent that there were two components in this small series. One comprised men from the southern part of the United States who had probably long

been infected with *Necator,* but who had earlier escaped detection or adequate treatment. As an illustration, one patient from whom 108 *Necator* in pure infection were recovered, was a native of southern Alabama, 19 years old, transported to Guam in January 1945, and when treated in July had been in service 11 months.

The second component included 5 men who had *Ancylostoma duodenale* only, their post-treatment worm counts being respectively 1, 2, 3, 5 and 47. These infections were in men mostly from northern United States, and their infections are all considered to have been contracted on Guam. The last 3 cases were proved to have been acquired on this island, through the handling of damp, unwashed bed linen at the civilian hospital during a period in the fall of 1944 when laundering facilities were inadequate and some soiled laundry accumulated for one or two weeks before washing. The fact that one of these men on routine examination had a high egg count, although he had not been beyond Guam, immediately put him under suspicion as probably having *Ancylostoma* secured on Guam itself. With this start Loughlin and Stoll (1946) were able to study these three cases in detail and establish the fact of fomite-borne ancylostomiasis.

Leyte-Returned Marines. As indicated elsewhere (Stoll, Loughlin, Harris and Chenoweth, 1946), the fact that a low incidence of hookworn infection—3 to 7 per cent—was the rule, organization to organization, in Guam garrison force personnel, threw into sharp relief the higher incidence—34 per cent—in marines who had participated in the Leyte operation and then returned to Guam for rest and retraining. The men were from artillery battalions and had had on Leyte their first Pacific duty west of the Hawaiian islands. Of 23 of these marines who were worm counted after treatment 87 per cent showed some *Ancylostoma,* although 82 per cent of the 1570 worms recovered from them were *Necator.* These men, who may be considered to have been exposed throughout the two months of the Leyte campaign and who were worm counted 6 to 8 months afterward, thus unwittingly brought back within themselves a sample for study of the hookworm species present in Leyte. From this sampling of infections acquired during combat operations it can be said that while *A. duodenale* may be regarded as well distributed on Leyte, it is the lesser component of the two species on that Philippine Island, in contradistinction to its status on Guam. These data are in harmony with the report of Leach, Haughwout and Ash (1923) who found 9 Bilibid prisoners from Leyte averaged 101 hookworms with 13 *Ancylostoma* each, with that of Manalang (1925) who recorded 93 per cent hookworm infection in Leyte, and with that of Tubangui, Basaca, Pasco and del Rosario (1935) who found 75 per cent of 169 Leyte residents positive for hookworm by the dilution counting method, with an average of 3850 EPG.

Natives of Okinawa. For the determination of the species index in Okinawans all the worms examined have been from necropsies. The largest number from a single case was 79, but 31 of the 41 necropsies are represented by less than 10 worms each. Of the total of 404 hookworms 70 per cent were *Necator americanus,* which was the only species encountered in over half of the cases.

HOOKWORM EGG SIZES

The fact that on Guam we were diagnosing infections in which either *Necator americanus* or *Ancylostoma duodenale* or both might be present, and the laborious

procedure required to determine the species status routinely by worm count, stimulated an inquiry as to whether hookworm egg sizes were sufficiently distinct to be of assistance in differential species diagnosis. It will be recalled that Stiles (1902) in his initial report on *"Uncinaria americana"* drew "Dr. Claytor's attention to the fact that the eggs did not agree with the measurements usually given for *Uncinaria duodenalis.*" Stiles followed with a specific diagnosis for each form in which he gave egg sizes of 52 by 32 microns for *"U. duodenalis"* and 64–72 by 36–40 microns for *"U. americana."* Textbooks in general encourage the idea that a rather clear-cut distinction exists between the eggs of the two species, at least in long diameter. Thus of 21 standard texts or reference works consulted two-thirds give the long diameter for *N. americanus* in the 64–76 micron range, *A. duodenale* as 56–61 microns. This opinion—that there is a size distinction—is not unanimous, however, some authors listing identical data for both species, some mentioning that they are practically indistinguishable although not giving data, some giving *N. americanus* ranges that overlap the 56–61 micron range for *A. duodenale.* (Thus, for the former Baylis, 1929, gives 53–66 microns; Castellani and Chalmers, 1919, and Hegner and Cort, 1921, give 58–80 microns; Chandler, 1929, gives 50–80, usually 70, microns. Darling, 1922, notes that there is practically no distinction, giving the long diameter of *N. americanus* as 72 microns, of *A. duodenale* as 70 microns.) For short diameter, most references agree that the range 34–40 microns includes both species, although Castellani and Chalmers (1919) and Hegner and Cort (1921) give 35–52 microns, and Baylis (1929) 28–44 microns for *N. americanus,* with Hoeppli and Khaw (1934) giving 34–38 microns for *A. duodenale.*

If the clear-cut break between a long diameter of 56–61 microns for *Ancylostoma duodenale* and 64–76 microns for *Necator americanus* were substantiated, foreknowledge of the presence of either species could be had by measuring an unselected sample of eggs during fecal diagnosis, and perhaps even the ratio of the occurrence of the two species in a given infection could be determined. To test this hypothesis eggs were measured from several individual worms, and several infected persons. From the latter *N. americanus* was the sole species later recovered by worm count after treatment in some instances; from others the post-treatment stools contained both hookworm species. Thus infections with differing species ratios had the opportunity of emerging with contrasting egg sizes.

A convenient method of making use of the idea of distinctive egg sizes would be to measure a fair sample of eggs in a slide preparation with the aid of an ocular micrometer. This method was employed for the measurements herewith presented, care being taken to prevent coverslip pressure on the eggs. With 12× ocular and 4 mm. objective (magnification 540 diameters), one ocular micrometer unit had a value of 2.92 microns. Measures were made to the nearest whole unit, giving the approximately 3 micron interval in the minimum, maximum and modal sizes shown in the tables. As Pearl (1923) indicated, such a scale interval would not affect the essential accuracy of the mean values secured from the measurements, and the modal values, it will be noted, do not differ from the means by more than 1 or 2 microns in most cases. This fact derives from the generally low variability of the measurements, most of the coefficients of variation being less than 5 per cent.

TABLE 2.—*Measurements in microns of hookworm eggs laid by females in vitro in 0.85 per cent sodium chloride solution*

Female	No. of eggs measured	Long diameter				Short diameter			
		Min.–Max.	Mode	Mean	S.D.	Min.–Max.	Mode	Mean	S.D.
A. duodenale ...	51	58–64	61	60.9	2.0	38–41	38	38.6	1.5
"	24	58–70	64	64.5	2.5	38–47	41	41.3	1.1
"	28	61–70	67	66.4	1.8	38–44	41	40.0	1.2
"	25*	50–72	60	57.7	5.0	41–44	41	42.1	1.3
"	25*	63–69	66	66.8	2.2	38–44	41	41.8	1.5
"	17*	57–69	60	61.9	3.3	38–44	41	42.1	1.8
"	13*	60–76	69	70.3	3.9	38–47	38	41.4	3.1
Total	*183*	*50–76*	*61*	*63.3*†	*4.7*	*38–47*	*41*	*40.6*†	*2.3*
N. americanus ..	18	67–73	70	70.5	1.3	38–41	38	38.1	0.1

* These 80 eggs were measured by W. D. Lindquist, CPhM, with 10 × ocular and 4 mm. objective, with 1 ocular micrometer unit equal to 3.15 microns; remaining measurements were made by the author with 12 × ocular and 4 mm. objective, with 1 ocular unit equal to 2.92 microns.
† Mean of means 64.1 × 41.0 microns.
S.D., Standard deviation.

Measurements were first made on fair samples of eggs laid *in vitro* in 0.85 per cent sodium chloride solution by live female worms rescued from post-treatment stools (Table 2). The long diameters of series of 13 to 51 eggs from 7 *Ancylostoma duodenale* females had means from 57.7 to 70.3 microns, whereas a series of 18 measurements on eggs laid by a *Necator americanus* female had a mean of 70.5 microns. This demonstration of overlapping size range of the two species is apparent also in their modal values (60–69 microns for *A. duodenale,* 70 microns for *N. americanus*), and the range of their minimum-maximum sizes 50–76 microns for the former, 67–73 for the latter). Similar lack of distinction occurred in the short diameters of the 201 above-mentioned eggs measured in physiological saline.

TABLE 3.—*Measurements in microns* of hookworm eggs in DCF examinations from 17 persons. The series are tabulated with reference to the number and percentage of* Necator americanus *recovered from the same persons in worm counts after treatment*

Group	Hookworms recovered after treatment		No. of eggs measured	Long diameter				Short diameter			
	Total	% *Necator*		Min.–Max.	Mode	Mean	S.D.	Min.–Max.	Mode	Mean	S.D.
A	71	100	11	64–70	64	66.6	2.0	38–44	41	40.9	1.1
A	70	100	24	58–70	64	63.7	3.6	38–44	41	41.2	1.7
A	29	100	41	61–79	67	69.1	3.4	35–41	38	38.6	1.4
A	22	100	32	58–76	64	66.0	3.9	38–44	41	40.5	1.7
A	17	100	15	61–67	61	63.4	2.4	38–44	41	41.0	1.6
A	7	100	7	61–67	67	65.5	2.1	38–44	41	41.5	1.7
A	3	100	10	58–64	64	62.6	2.3	38–41	38	39.3	1.3
A	1	100	15	55–64	61	61.3	2.4	.....	..	...	..
Total		...	*155*	*55–79*	*64*	*65.5*†	*4.2*	*35–44*	*41*	*40.1*†	*1.4*
B	169	99	15	58–70	67	63.6	2.9	38–47	41	41.4	1.1
B	137	98	17	61–70	64	65.6	2.3	41–44	41	42.2	1.5
B	388	96	15	58–70	67	65.0	2.2	41–44	41	41.1	0.7
B	371	96	15	61–70	67	66.0	2.3	38–41	38	39.3	1.0
B	371	96	50	61–70	67	66.4	2.2	.....	..	...	..
B	78	82	15	55–70	64	63.7	3.7	38–44	41	40.3	1.4
Total		...	*127*	*55–70*	*67*	*65.4*†	*2.4*	*38–47*	*41*	*40.9*†	*1.8*
C	190	44	15	58–70	67	65.1	3.8	38–44	38	38.9	1.6
C	7	43	15	58–64	61	61.6	1.0	38–44	41	40.0	1.9
C	31	29	16	61–67	64	65.4	1.7	35–44	41	39.7	1.4
C	31	29	25	58–76	64	65.2	3.9	.....	..	...	..
C	3	0	10	58–64	61	60.9	1.8	35–47	41	40.3	1.0
Total		...	*81*	*58–76*	*67*	*64.0*†	*3.3*	*35–47*	*41*	*39.7*†	*1.4*

* Eggs were measured with 12 × ocular and 4 mm. objective (resulting magnification 540 diameters), with one ocular micrometer unit equal to 2.92 microns. This establishes the approximately 3 micron interval in the minimum-maximum and modal values.
† Mean of means for A, 64.8 and 40.4 microns; B, 65.1 and 40.9 microns; C, 63.6 and 39.7 microns.
S.D., Standard deviation.

In Table 3 are presented measurements of fair samples of 7 to 50 eggs from 19 DCF preparations, using saturated sodium chloride solution of specific gravity 1.200 for floatation. The fecal specimens examined were from patients from 8 of whom (A, Table 3) only *Necator americanus* were recovered after treatment; from 5 others (B, Table 3) 82 to 99 per cent of the worms were *Necator;* and from 4 others (C, Table 3) 0–44 per cent. Egg measurements in long diameter summarize as follows:

		Mean	*Mode*	*Minimum–Maximum*
A.	100 per cent *Necator*	61.3 – 69.1 μ	61 – 67 μ	55 – 79 μ
B.	82–99 per cent *Necator*	63.6 – 66.4 μ	64 – 67 μ	55 – 70 μ
C.	0–44 per cent *Necator*	60.9 – 65.4 μ	61 – 67 μ	58 – 76 μ

Similar lack of distinction occurred in the short diameters of these 363 eggs, as may be noted in Table 3.

This lack of effective size distinction between Group A or B, and C is particularly of interest in that with the 2 to 3 times greater egg laying capacity characteristic of *Ancylostoma duodenale* (see Soper, 1927) the percentage of *Necator* eggs appearing in the stools would be reduced in Group B to about 70 to 97 per cent, and in Group C to about 0 to 27 per cent.

It is obvious therefore that while worm counts after treatment demonstrated *Necator americanus* was present in different ratio in the A, B and C groups, similar distinctions were not revealed by egg measurements made at pre-treatment fecal examinations. The essential conclusion is therefore identical with that of Darling (1922), although our data enlarge his statement of sizes that the eggs of *N. americanus* at 72×36 microns "are nearly the same as *A. duodenale*" at 70×40 microns.

The facts obtained by measuring several hundred eggs thus afford little encouragement to the possibility of differential speciation of *Necator* and *Ancylostoma* from egg sizes encountered in diagnostic preparations. This accords with a decision of Schwartz and Tubangui (1922) in Manila who "after numerous measurements of hookworm ova" decided "no definite conclusions as to the relative abundance of *Ancylostoma* and *Necator* in the Philippines can be drawn from our data" since "the size of eggs of the two species of hookworms commonly parasitic in man doubtlessly overlap." Reservation needs to be recorded, therefore, as to the validity of a classification included in a report by Lowe and Lancaster (1944) of the types of hookworm identified from the ova obtained from soldiers who served in the islands north of Australia. The specific criteria employed were not mentioned, but of 29 such patients, 12 are tabulated as cases of *A. duodenale,* 2 of *A. braziliense,* 21 of *N. americanus,* and 6 of mixed *N. americanus* and *A. duodenale.* It would seem especially unfortunate to have records incautiously disseminated (as in Helm. Abstracts, 1944, **13**: 12 and Trop. Dis. Bull., 1944, **41**: 950 and 1945, **42**: 667) of the occurrence of adult infections of *A. braziliense* in man on the basis of indirect evidence of this character.

SUMMARY

1. In Guam, 76 per cent of the adult hookworms obtained from natives were *Ancylostoma duodenale,* which constituted pure infections in about two-thirds of the cases worm counted.

2. Garrison force personnel on Guam were found on worm count after treatment to have either *Necator americanus* or *Ancylostoma duodenale*. Evidence indicated that the former were infections contracted in the southern United States, the latter in Guam.

3. Of marines who had been in the Leyte, P. I., operation for two months and returned to Guam in late December, 1944, those infected with hookworm and treated in mid-1945 harbored *Ancylostoma duodenale* in 87 per cent of the cases, but 82 per cent of all worms recovered were *Necator americanus*. This is in harmony with somewhat fragmentary earlier Philippine reports that hookworm is widespread in Leyte, includes both species but predominantly *Necator*.

4. Hookworms obtained from necropsies of Okinawa natives indicated that over half of the infected individuals harbored *Necator americanus* only, the species constituting 70 per cent of all worms examined.

5. Measurement of hookworm eggs in connection with fecal diagnosis by salt floatation did not reveal reliable size differences between the two species as compared with the species index of hookworms recovered after treatment of the infected donor individuals.

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THE INCIDENCE OF FILARIASIS IN THE CENTRAL PHILIPPINES[1]

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The data reported herein represent the result of a survey of the incidence of filariasis among the native populations of the San Pedro Bay (Leyte Gulf) area of the central Philippines. A greater proportion of the work was done in Salcedo and Mercedes on southern Samar during March and April of 1945. A single survey on Leyte was conducted at Tolosa.

Blood smears were taken on volunteers in each of the villages surveyed. Care was exercised to insure a representative sampling of both sexes of all age groups. All results are based on the examinations of a single stained dehemaglobinized thick blood smear taken from each native volunteer at the time indicated. To obtain information on periodicity as well as incidence, surveys in one area were conducted at midday as well as at night.

Detailed results of these surveys are given in Table 1. All microfilariae found were identified as *Wuchereria bancrofti.*

TABLE 1.—*Incidence of circulating microfilariae in natives of the central Philippines*

Locality	Time of examination	Number of natives examined	Per cent positive	Microfilariae per positive slide
Tolosa, Leyte	Night*	253	2.8	8.4
Salcedo, Samar	Night	300	1.3	3.5
Mercedes, Samar ..	Night	436	9.6	27.7
Do	Day†	353	3.4	8.6

* The night smears were taken between 9 : 30 and 11 : 30 PM.
† The day smears were taken between noon and 1: 30 PM.

These data indicate a low incidence of microfilariae in the areas surveyed. Phalen and Nichols (1909) reported an incidence of 2.0 per cent in Tolosa and 2.8 per cent among 141 natives from the island of Samar. As may be noted in Table 1, the blood smears taken at Mercedes revealed a higher incidence at night than at midday, thus indicating a nocturnal periodicity. However, the incidence in this area at noon was much higher than is usually encountered in a nocturnally periodic area at midday. Africa, Garcia and Layco (1935) reported this modified periodicity in the Philippines. Although their findings are correct, their explanation, based on the ethnological origin of the Philipinos, is probably faulty. They believed this form to be the result of the mixing of the nocturnal strain from Asia and a non-periodic strain introduced by native migrations from New Guinea and the Celebes. However, *W. bancrofti* in these latter islands has a definite nocturnal periodicity. Ashburn and Craig (1906) previously considered the microfilariae in the Philippines were the non-periodic strain.

The average number of microfilariae per positive slide for the day and night

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[1] The opinions expressed herein are those of the author and are not to be considered as official or reflecting the views of the Navy Department or the Naval service at large.

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surveys are included in Table 1. Not only was the incidence of circulating microfilariae lower during the day in the Mercedes area but the number of microfilariae per volume of peripheral blood was lower. Although an attempt was made to make all blood films of comparable size, it is realized that these microfilarial counts are not absolute values.

The surveys reported herein indicate a low incidence of microfilariae among the native population of the area. The strain of *W. bancrofti* encountered exhibited a modified nocturnal periodicity resulting in a very low incidence of circulating microfilariae at midday and a relatively higher incidence at night.

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AËDES ATROPALPUS (COQ.) A NEW MOSQUITO VECTOR OF *PLASMODIUM GALLINACEUM* BRUMPT

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In 1936, Brumpt succeeded in transmitting *Plasmodium gallinaceum* to the domestic fowl by bites of infected *Aëdes aegypti* (= *Stegomyia fasciata* (Linn.)). Subsequently, 27 other species of mosquitoes (17 *Aëdes,* 5 *Armigeres,* 3 *Culex,* 1 *Mansonia,* and 1 *Culiseta*) have been shown to be definitive hosts of the parasite, but not all have been proved capable of actual transmission (Brumpt, 1936b; Roubaud et al, 1939; Vargas and Beltran, 1941; Russell and Mohan, 1942; Russell and Menon, 1942; Cantrell and Jordan, 1945; Paraense, 1945). Of the ten susceptible species occurring in North America, viz., *Aëdes aegypti, A. campestris* D. & K., *A. stimulans* (Walk.), *A. vexans* (Meig.), *A. triseriatus* (Say), *A. trivittatus* (Coq.), *Culex quinquefasciatus* Say, *C. salinarius* Coq., *Mansonia perturbans* (Walk.), and *Culiseta inornata* (= *Theobaldia inornata* (Will.)), only the first three are recorded as having transmitted *P. gallinaceum* experimentally.

In 1941, *A. atropalpus* was shown by Laird to be susceptible to the avian parasite *P. lophurae,* but transmission to ducks was not accomplished although "great masses of sporozoites" were found in the salivary glands.

The present note describes the infection of *A. atropalpus* with *P. gallinaceum,* compares the incidence and intensity of the infections in this species with those observed in *A. aegypti,* and records the transmission of the infection by *A. atropalpus* to the domestic fowl.

Laboratory-reared *A. atropalpus* (Trembley, 1945) were used in these studies. Due probably to a characteristic which allows this species to lay viable eggs without blood meals (unpublished manuscript of the author), only a small proportion of the females in a cage seek blood when it is offered. In the beginning, it was found necessary to expose several hundred mosquitoes to an infected chick in order to get 30 or 40 to feed. This difficulty was later overcome by the simple procedure of placing one's hand in the cage to attract the "hungry" females and when they alighted immediately transferring them to individual containers. About half of these selected mosquitoes fed promptly when offered a blood meal. After the mosquitoes had engorged, the containers were placed on saturated towels in a constant temperature room maintained at 28° to 30° C and approximately 80 per cent relative humidity.

In preliminary studies, dissection of 53 *A. atropalpus* taken from 12 different lots revealed 45 infections; the comparatively high individual oöcyst counts suggested a greater intensity of infection than ordinarily encountered in *A. aegypti.* A more critical study of this difference was made by comparing lots of the two species fed in succession on the same infected chick. Five such tests were carried out, the essential details being as follows: about 100 *A. aegypti* were allowed access to an infected chick through the bobbinet top of their container, and the engorged females removed to another container by means of a suction tube. As soon as the

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A. aegypti had fed, the *A. atropalpus* were applied singly. The care of the species after feeding differed only in that a more humid microclimate was provided for the *A. atropalpus*.

Incidence of infection in the corresponding lots of *A. atropalpus* and *A. aegypti* in the five tests was determined by examination of midguts, salivary glands, or both, in equivalent samples, as shown in Table 1.

TABLE 1.—*Incidence of infection in corresponding lots of* A. atropalpus *and* A. aegypti

Test No.	No. of mosquitoes	*A. atropalpus*		*A. aegypti*	
		Pos.	Per cent	Pos.	Per cent
1	15	14	93.33	9	60.00
2	19	18	94.73	8	42.10
3	12	12	100.00	8	66.66
4	22	15	68.18	12	54.54
5	19	19	100.00	19	100.00
Total	87	78	89.65	56	64.36

Of the 87 *A. atropalpus* mosquitoes examined, 78, or approximately 90 per cent, showed infection, while of the 87 *A. aegypti,* 56, or approximately 64 per cent were positive.

The intensity of the infection in *A. atropalpus* and *A. aegypti* was determined by a comparison of the oöcyst counts in corresponding lots. Midguts from 87 mosquitoes of each species were examined, but accurate counts were not possible after the oöcysts had begun to rupture, therefore only 37 mosquitoes of each species are compared in Table 2.

TABLE 2.—*Intensity of infections in corresponding lots of* A. atropalpus *and* A. aegypti

Test No.	No. of midguts	Number of oöcysts			
		A. atropalpus		*A. aegypti*	
		Range	Mean	Range	Mean
1	8	21 to 399	174.75	0 to 2	0.75
2	6	6 to 25	13.16	0 to 13	5.66
3	13	0 to 50	9.07	0 to 8	1.76
4	10	1 to 479	254.70	1 to 131	29.80
Total	37	0 to 479	111.94	0 to 131	9.75

A greater intensity of infection as evidenced by the number of oöcysts was noted in *A. atropalpus*. There was a significant difference in the means, *A. atropalpus* showing 111.94 ± 23.43 (standard error of the mean), *A. aegypti* 9.75 ± 3.98.

Transmission by *A. atropalpus* was attempted on 26 chicks. The chicks were two days old and weighed about 40 grams; the method of taking blood smears, counting parasites, and interpreting data followed the procedure established for the evaluation of *P. gallinaceum* infections transmitted by *A. aegypti* (Coatney et al, 1945). The parasite was transmitted by *A. atropalpus* through three successive generations. The first series consisted of 3 chicks; 2 were inoculated with glands from 2 positive mosquitoes and one was bitten by 2 mosquitoes and then injected with their infected glands. The second series was composed of 7 chicks; 2 were bitten by infected mosquitoes and 5 injected with positive glands. The third series included 16 birds; 9 were bitten and 7 injected. Of the 26 chicks exposed, 3 died of undetermined cause before the time of expected patency, and of

the remaining 23, 20 developed infection. The course of infection in these chicks followed essentially the same pattern as that described by Coatney and coworkers (1945) where the transmission was effected by *A. aegypti.*

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TSUTSUGAMUSHI DISEASE (SCRUB OR MITE-BORNE TYPHUS) IN THE PHILIPPINE ISLANDS DURING AMERICAN RE-OCCUPATION IN 1944–45. II. OBSERVATIONS ON TROMBICULID MITES

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In a previous paper, the authors (Philip, Woodward and Sullivan, 1946) reviewed the experience with scrub typhus in the American armed forces during action in the Philippine Islands in 1944–45. The hospital, field and laboratory studies by various Army and Navy agencies contributing to those observations produced the first incontrovertible evidence of the presence of the disease in the Archipelago. A restricted report on this subject was previously published by us in the December 1945 number of the Journal of Military Medicine in the Pacific.

It was not possible in the first report to elaborate the observations on trombiculid mites which here are presented together with a key to facilitate the work of future investigators as studies on the problem are intensified in this area. Acknowledgments were tendered in that report and will not therefore be repeated in this supplemental discussion.

The mite fauna of the Philippines is unquestionably a rich one, and many species will be recorded as the host sampling is expanded. The present study was necessarily limited by time and circumstances to a far from exhaustive, rapid checking of mites parasitic on rats in a few focal areas where cases of scrub typhus were contracted in troops.

In addition to *Trombicula akamushi* and *T. deliensis* which are known readily to attack man, only one other Philippine species at present has been observed to attack man, namely, *T. wichmanni* discussed below. On the basis of the reports by Gater (1930) that *T. acuscutellaris* has been taken off persons in Malaya, it also should be a human parasite in the Philippine Islands. Since the last two are not infrequent parasites of rats, are widely distributed in the Philippine Archipelago, and are known to attack man, we feel that their rôle as possible incidental vectors of scrub typhus should be investigated. No reports were received of mite-caused scrub-itch in troops similar to that experienced in New Guinea and northern Australia.

T. akamushi is the classical vector in Japan as demonstrated by several groups of Japanese investigators, and its synonym or variant, *T. fletcheri,* was recently incriminated in New Guinea (Blake et al, 1945, Kohls et al, 1945). *T. deliensis* has been suspected circumstantially as a transmitter in Malaya, Sumatra and other places, and it was first confirmed as a vector (under its synonomic name, *T. walchi*) in recent studies on Bat Island (Kohls et al, 1945, Philip and Kohls, 1945).

As collections continue to accumulate from various areas of the Orient and Far Pacific the dividing line between the two known vector species *Trombicula akamushi* and *T. deliensis* becomes less clear on purely morphological grounds.

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THE AKAMUSHI-DELIENSIS COMPLEX

In the Philippines, specimens referable to what has been considered typical *T. deliensis* have been studied by us from the focal areas on southern Mindoro (Figs. 3 & 4), southern Samar (Figs. 1 & 2), Negros, and Mabitae, central Luzon. Hosts have been the rats, *Rattus mindanensis, R. rattus umbriventer, R. vigoratus,* and *R. calcis,* and shrews, *Crocidura occultidens.* The first was the most heavily infested in the areas studied, while the smaller *R. vigoratus* showed less parasitism and more tendency to invade military installations and premises of local residents. *R. umbriventer* was recently described from among rats collected for us by the 31st Malaria Survey Detachment on Mindoro and its importance and distribution remains to be worked out.

FIG. 1. Undergrowth in a neglected coconut plantation adjoining military installations where scrub typhus occurred near Guiuan, Samar, providing good rat and mite cover.

Mites we have identified as *T. akamushi* have been seen from northern and central Luzon and Negros, collected off the rats, *R. mindanensis* and *R. calcis,* and shrews. Wherever it has occurred, variants from the standard form have also been taken which deviated toward *T. fletcheri* on the one hand, and toward *T. deliensis* on the other. Both species were reported by Captain Mohr (ident. by Womersley) from Dagupin, Lingayen area, Luzon from *R. r. alexandrinus.*

Mites from rats and shrews taken at Bagabag, northern Luzon, have been particularly variable in the dorsal setal count, and standard data of the scutum. While we have not attempted biometric analysis, lines of cleavage for separation of subspecies and even of *T. deliensis* have not been evident on morphological grounds. Adventitious, or irregular setae appear particularly in the sublateral, posterior position on the dorsum to confuse the DS counts, and have been seen in or between every

FIG. 2. Rough coralline floor in plantation exposed by flame thrower during anti-typhus control activities near Guiuan, Samar.

row except the first or humeral pair. By including such irregular setae in the rows immediately behind their location, variation in DS of 173 specimens is shown in Table 1.

TABLE 1.—*Variation in numbers of setae in dorsal rows of 173* T. akamushi, *Bagabag, Luzon*

DS row No.	1	2	3	4	5	6
Extreme count	2	8–11	6 9	6–8	4–6	2+

In all there were 30 different combinations observed including typical *deliensis* but the four commonest were 2–8–8–8–4–2 (40 times), 2–10–8–8–4–2 (23), 2–9–8–8–4–2 (21), and 2–9–8–7–4–2 (18). Even so, elaboration of setae on the posterior dorsum in this population is not as marked as in *T. akamushi* we have studied from Japan and might be considered by some acarologists as variants of a population of *fletcheri*.

Extremes in micrometric standard data for the dorsal scutum of 50 mites in 6 different animal lots taken in the Bagabag area are given in Table 2.

TABLE 2.—*Standard data in* μ *of dorsal scuta of mites taken on rats and shrews at Bagabag and Mercia, compared to* akamushi *taken on mice in Japan, and to* fletcheri *and* deliensis *as given by Womersley (1944)*

Mites	No.	AW	PW	SB	SD	PSB	A–P	AM	AL	PL	SENS
Bagabag L.	50	58–64	66–76	26–31	35–45	12–16	21–31	45–54	29–41	48–58	48–72
Mercia, N.	25	53–66	60–76	21–30	33–38	12–13.5	22–29	38–47	33–35	43–55	46–62
Japan	32	56–68	68–77	25–32	36–43	12–17.5	24–32	49–56	37–46	49–66	52–64
fletcheri	13	62–72	73–79	30–36	34–44	.....	25–32	45–56	35–40	43–57	62–68
deliensis	53	60–72	70–93	26–36	35–47	.....	25–32	47–72	40–57	50–94	60–72

Comparable variations also have occurred in small series of specimens we have been privileged to study from Ceylon (Radford)[1] and Burma (USA Typhus Commission). The situation with regard to this mite-complex in the Philippines illustrates what is coming to be emphasized in the larger experience in other localities, namely, that in certain geographic locations the respective mite populations of *akamushi* and/or *deliensis* are relatively constant and usually readily assignable by known taxonomic standards, whereas in some localities including the classical endemic areas in northwestern Honshu, the characters become mutable, and very confusing. This has been noted by various Japanese workers, and Miyajima and Okumura (1917) reported as few as 24 total dorsal setae, fewer than usually seen

FIG. 3. Catching rats in a field of young, volunteer "talahib" grass (*Saccharum spontaneum*) in an abandoned cane field on Mindoro, Philippine Islands, where cases of scrub typhus were contracted by American troops in combat. Mature clump silhouetted against tree in back-ground.

in *deliensis*. In the absence of adequate discussion of the nymphs and adults in the literature, and of adequate series of specimens as yet for study, it is not known how much variation may occur in characters of the crista which are now considered to differentiate between these stages of *akamushi* and *deliensis,* but Miyajima and Okumura figured similar differences even between the nymph and adult of reared *akamushi* alone.

Variations could not be correlated with different types of environment. Uncomplicated colonies of *deliensis* were found in grassy fields (*Saccharum spontaneum*) on Mindoro (Figs. 3 & 4) and under coconut canopies on Samar (Figs. 1 & 2). Mixed *deliensis* and *akamushi* occurred in varying vegetational cover including both grassy pastures and tree shade on Luzon (Fig. 5), and in "kogan" grass (*Imperator cylindricus*) on mountain ridges on Negros.

It appears that these species may belong in a complex of recent evolutionary origin. While isolated geographic populations may be tending toward subspeciation,

[1] In a paper just published, Radford (1946) does not mention any but standard *deliensis* among Ceylonese collected and reared specimens.

FIG. 4. Burrows of rats in "talahib" grass. These were dry and shallow, but the rats carried some *T. deliensis* and many *A. philippensis* and *A. kohlsi* in this area near San Jose, Mindoro.

until adequate biological grounds are available for substantiation, it appears to us that attempts to break it down into smaller taxonomic units at this early stage will only serve to confuse rather than clarify the problem for medical and other practical

FIG. 5. Rat burrow among grass at base of banana plant, Bagabag, Luzon, from which were trapped several *R. mindanensis* and shrews with heavy infestations of mites.

purposes, as illustrated by the Bat Island studies of "*T. walchi.*" Gater (1930) and others have also pointed out these systematic difficulties which unfortunately have involved one of the most important groups of mites. In graphic analysis of DS counts of 202 specimens he produced a bimodal frequency curve, the *deliensis* group with an average of 28 total setae (22–31) and the *akamushi* group with an average of 34 (31–40). This was reflected also in host habits, *deliensis* being found commonly on rats, comparatively rarely on man, and *akamushi* reversing this host preference; the common Malayan host of the latter would need to be found to substantiate this difference in selectivity, however.

It could be argued that it is as logical to maintain a group in the higher DS count brackets (2, 10, etc.) under *T. fletcheri,* but mites in the same series and often more abundant with count, 2, 9, etc., will embarrass assignment to one rather than the other, as more than purely arbitrary (the count 2–9–6–6–4–2 could as easily be a variant of *deliensis* as 2–7–6–6–4–2 which also occurs). Again it could be argued that the Bagabag series as a whole show more reduction in posterior rows in better keeping with *fletcheri,* but in the absence of rearing, and other supportive data, we are not convinced that this is more than a local racial tendency in view of intergrades seen in other localities. The original description of *fletcheri* shows about double the usual number of ventral setae encountered on these specimens.

It is especially difficult when one attempts to dichotomize even *akamushi* and *deliensis,* as admittedly arbitrarily handled in our key. It easily could be that *deliensis* merits at most only subspecific standing. We prefer to leave to specialists attempted further subdivision until present inadequate information is improved.

DATA ON OTHER SPECIES OF MITES

Where collections were by others than ourselves credit is given in parentheses. Identifications were by us, or checked by us where so indicated.

Trombicula wichmanni. Taken on Luzon at Bagabag from *R. mindanensis,* and *T. calcis,* and at Mabitae from *mindanensis* (Morley, identified by Straube and by Philip); on Mindoro at San Jose from *R. mindanensis;* on Negros near Mt. Mandalagan on *mindanensis* (Christenson). Engorging specimens were easily distinguished among patches of *deliensis* and *akamushi* in the host ears as contrasting bright orange and more rounded in outline.

The species has previously been reported from man in North Celebes (Womersley and Heaslip, 1943) and in Malaya (Gater, 1930). It also was taken from one of several mite-bite papules on one of us that developed after return from rat-trapping at Bagabag.

The correct specific name for this Philippine representative of Ewing's *Eutrombicula* is still in question and cannot be assigned with confidence until acarologists have settled the status and synonomy of several other names including *T. hirsti.* Wharton (1946) briefly characterized the minor genera, *Eutrombicula* and *Acariscus;* he also has called attention to the similarity of the Standard Data of this to *T. buloloensis,* one of the mites causing scrub-itch in New Guinea, a condition not encountered in the Philippines to our knowledge though *T. wichmanni* has been present in areas on at least three islands when troops presumably were exposed. We have preferred to retain this older name pending taxonomic clarification. It may be pointed out that Gunther (1940) believed on epidemiological grounds that

T. buloloensis (under the name *T. minor*) was the important mite vector of scrub typhus in New Guinea, and Wharton (in press) reports it as the only species found on Ponam Island 3 months after several cases of scrub typhus had occurred in Naval personnel and control work had been intensive.[2] Probably this was the species responsible for the complaints of scrub-itch among early visitors and expeditions along the North coast of New Guinea lucidly summarized by Oudemanns (1906), who blamed *T. wichmanni* on circumstantial grounds.

Because of the possible importance of *T. wichmanni,* we append here certain systematic data on our Philippine material for benefit of later students. The standard data are given in Table 3.

In this series the dorsal and ventral setal counts are remarkably constant though we have observed the same shift of the outside pair on row 4 in unfed specimens to the venter in fed specimens as early described by Oudemanns, DS = 2, 6(7), 6, 4(2), 2 and VS 2, 2, 6, 2, 4(2), 2. Part of a series of specimens from Bougainville Island by Wharton vary in the second and fourth dorsal rows by one or two setae more than in our series emphasizing the closeness or possible identity of

TABLE 3.—*The extremes of variation in standard data in* μ *of the scuta of 22* T. wichmanni *from Bagabag, Luzon (A) compared to* T. wichmanni *from Brit. N. Borneo (B) and* T. "minor" (buloloensis) *from New Guinea (Womersley, 1944) (C)*

	No.	AW	PW	SB	SD	PSB	A–P	AM	AL	PL	SENS
A.	22	79–93.2	93–105	44–44	57–66.5	30–36.5	33–40	42–49	45–56	52.5–56	52–60
B.	4	76.5–80	91.5–93	38–40	48–52	25–27	31.5	43	46–48	58–59	63
C.	73	75–91	90–106	40–47	61–72	.....	31–39	34–50	40–58	47–64	50–68

wichmanni and *buloloensis*. Oudemann's original *wichmanni* came from a Goura pigeon on the Vogelkop Peninsula of Dutch New Guinea (mislabelled Java in some literature), which further strengthens the likelihood of synonomy of Gunther's *buloloensis* originally described from the Wau district of British New Guinea. Specimens identical to ours have been taken as far West as Burma (Fuller, USA Typhus Commission).

Trombicula acuscutellaris. Taken on Luzon, at Bagabag from *mindanensis,* and at Mabitae from rats (Morley, identified by Straube and by Philip) ; on Mindoro at San Jose from *mindanensis* and *umbriventer;* and on Negros near Mt. Mandalagan from *mindanensis* (Christenson). Gater (1930) reports it as a human parasite in Malaya. It was less frequently encountered on rats in the Philippine Islands than the preceding species, though in the same areas.

Trombicula bodensis. This species was described in 1940 from the mouse deer in British North Borneo. A specimen from a rat at Mabitae (Morley) was identified by Ensign Straube, and checked by us.

Trombicula scincoides. A common parasite on skinks and lizards on Guiuan, Samar, and on Mindoro. There are no records that it attacks other than cold-blooded hosts, as in New Guinea.

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[2] In this connection the remarks of McCulloch (1944, Med. J. Austral., p. 544) are pertinent: "At Dumpu (Ramu Valley), however, *Schöngastia blestowei* alone was more clearly indicated as the vector (as well as the cause of itch) . . . in several camps with a history of typhus it alone was found after most careful searching." Just received is another article by the same author (Med. J. Austral., May 25, 1946) containing the pertinent statement "It (*T. wichmanni*) was the only species found in the country near Balikpapan where scrub typhus infection existed, and so must be suspected as a potential vector of the disease."

Trombicula n. sp. In addition to the above, an apparently undescribed species near *T. rara* was taken off skinks in the foothills above San Jose, Mindoro. It has not been included in the accompanying key.

Heaslipia (*Trombiculoides*) *gateri.* Originally described in 1943 from rats in Perak, this interesting and little known species was taken on rats in a restricted area near Progresso, Mindoro, in February 1945 by Captain Mangrum of the 38th Malaria Survey Detachment. No other specimens were encountered by us in later surveys.

Ascoschöngastia indica. Previously recorded as widespread on rats from Calcutta, India, to Northern Queensland, Australia, this species has been found in some abundance in the ears of "domestic" rats (*alexandrinus* and *norvegicus*) in Manila. It is the only urban trombiculid we have encountered in the Philippines. Gater (1930) mentions a specimen each of 3 (unstated) species taken on urban rats in Malaya that also attacked man. *A. indica* is not known to do so. The nymph and adult were recently described by Radford (1946).

At least in Manila it has assumed the peculiar habit of attaching not only in the outer ear conchae of its rat hosts, but along the whole external auditory canal. It has not been found to attack man but its possible rôle in natural maintenance of endemic (murine) typhus should be investigated since Dove and Shelmire (1931) have shown that the Tropical rat mite, *Liponyssus bacoti,* can transmit the disease. One attempt by us (Woodward, Philip, and Loranger, 1946) to demonstrate infection in *A. indica* off an OX19 positive rat in Manila proved negative.

Other Luzon records: Lingayen area off *calcis* (Mohr), Bagabag from *mindanensis,* and Mabitae. This species often has the peculiar character of the 4 middorsal setae in row 2 forming a concave rather than the usual convex line, and a forward shift of the lateral setae giving the appearance of two pairs instead of one pair of humeral setae (row 1).

Ascoschöngastia philippensis. This was described by us (1946) from both *mindanensis* and *vigoratus* taken in the San Jose area on Mindoro. In some localized spots it was numerous, and the dominant or only mite species taken on rats. Reported also by Womersley among material received from Lingayen by Mohr off *R. r. alexandrinus.* No human parasitism by this or the following species was reported. While the descriptions of these two species were in press a paper appeared by Ewing (1946) necessitating a change in generic assignment from the former conception of *Neoschöngastia.*

Ascoschöngastia kohlsi. This like the above was described from Mindoro rats where some other local populations carried it as the predominant mite parasite in the ears.

The above list of Philippine mites reflects preliminary emphasis on sampling from rats as hosts. The only other records of Philippine mites which we have are in the U. S. National Museum. *Trombicula piercei* was described by Ewing (1931) from *Hipposideros* bats from Sagay, Occidental Negros. The types are too distorted to permit inclusion in our key, but any mites with the sensillary pores situated almost on the rear margins of the scutum and less than twice the diameters between them may be suspected as this species. Unpublished records of Ewing also include *Eutrombicula hirsti* (what we have referred to above as *T. wichmanni*) from man and chickens, and two undescribed *Trombiculas* from the above locality, and a *Eutrombicula* "near *hirsti*" from Mindoro. Wharton has an undescribed

Neoschöngastia (ex-*Paraschöngastia* of authors) from the kingfisher and dollar bird on Samar,[3] which is the first known record of the genus in the Philippines. It is likely that *N. gallinarum* will also be found on some avian host there also.

As collecting is intensified in the Archipelago not only will other species doubtless be taken on rats, but many additional described and even undescribed species will be found on other vertebrate hosts.

To enable preliminary familiarity of non-specialists with the known or suspected Philippine mite fauna, particularly Army medical and sanitary personnel concerned on the problems of mite-borne disease, the following key is offered. It is not intended as an exhaustive analysis, and for identification of important material or species that will not run in the key, specimens should be sent to specialists. Some species have been included which occur on neighboring islands, or whose parasitism of migratory hosts may bring them into the Philippines. Symbols are illustrated in Fig. 6. Starred species (*) indicate actual Philippine records known to us.

FIG. 6. Systematic symbols as applied to the scutum of *Ascoschöngastia philippensis* with inflated (globose) sensillae. Photographed with oblique lighting by Mr. E. Parrish of the Animal Health Laboratory, Sydney, NSW, Australia.

KEY TO KNOWN OR POSSIBLE PHILIPPINE TROMBICULID MITES

1. Scutal shield with entire margins behind the anterior angles evenly rounded, and with eleven setae or bristles ..... *Heaslipia gateri**
   Scutum with more or less well-defined postero-lateral angles and with never over eight setae, the lateral ones restricted to two pairs 2
2. Scutum with a pair of anterior median (AM) setae immediately behind a small, forward-directed, blunt process on anterior margin. *Leeuwenhoekia australiensis*
   Scutum without such a process and normally only one AM seta 3
3. The pair of sensillary setae (SENS) situated medially in the scutum in large pores are hair-like ..... (*Trombicula*) 7
   SENS distally swollen, globose or paddle-shaped 4

[3] Description of this has just appeared in this Journal (June) as *N. riversi*, p. 299.

4. Scutum with a transverse carina or ridge across the sensillary pores; all marginal setae, short, stout, subequal in length and densely ciliate (on fowls in Malaya and Formosa) ..... *Neoschöngastia gallinarum*
   Scutum normal on the disc, or longitudinally depressed between the pores; postero-lateral setae (PL), slender, much longer than AM. (*Ascoschöngastia*) 5
5. SENS paddle-shaped; antero-lateral setae (AL) of scutum much shorter than AM and PL ..... *A. indica**
   SENS globose; AM much shorter than AL and PL 6
6. Scutum with sinuous posterior margin, the SENS widely separated, AM relatively slender ..... *A. philippensis**
   Scutum with evenly curved rather indistinct hind margin; SENS very close, separated by about the diameter of the pores, AM stout and densely ciliated. *A. kohlsi**
7. Scutum plainly pentagonal with distinct median angle posteriorly; dorsal setae (DS) behind scutum about 60 in number ..... *T. ascuscutellaris**
   Scutum not produced behind into a median, apical angle, DS usually much fewer 8
8. Scutum sub-rectangulate, PL situated well ahead of postero-lateral angles (Sumatra) ..... *T. keukenschrijveri*
   Scutum often with rounded or irregular hind margins, when straight PL always in rear corners 9
9. DS relatively few, 20 to 22 in number usually arranged in transverse rows of 2 6 6 4 (6) 2; SENS shorter than AW (distance between bases of AL) 10
   DS 26 or more; SENS usually as long as or longer than AW 14
10. Scutum large, sensorial pores much nearer AL than PL ..... *T. (E.) rara*
    Scutum of average size, SENS situated as near or nearer PL than AL 11
11. Posterior margin of scutum an even curve (Formosa) ..... *T. hatorii*
    Posterior margin not on even curve, flattened mesally 12
12. Cold-blooded hosts, especially lizards; SENS short, but little longer than the distance between their bases (SB) ..... *T. scincoides**
    Warm-blooded hosts; SENS much longer than SB. *T. (Eutrombicula) wichmanni**
13. DS totalling less than 60, often with one or no rows of 10 setae 14
    DS with three rows each with 10 or more setae, total DS 68 (on birds, Formosa) ..... *T. corvi*
14. Scutum small, SENS subequal in length to PW and at least twice the scutal depth (DS 2 8 6 6 6 4) ..... *T. bodensis**
    Scutum normal in size, SENS shorter than PW and less than twice the scutal depth (DS usually otherwise) 15
15. Scutum with upper and lower margins forming even, shallow, convex curves, DS 26 (Formosa) ..... *T. issikii*
    Scutum never with both margins evenly curved 16
16. Total DS usually more than 30 with more than one row of 8 or more. *T. akamushi**
    Total DS normally 28, arranged 2 8 6 6 4 2 ..... *T. deliensis**

ANTI-MITE CONTROL MEASURES DURING RE-OCCUPATION

In connection with the discussion of case occurrence and foci of scrub typhus in troops presented in the first report (l.c.), it is of interest to review briefly the circumstances in mite control measures in certain operations.

Most troops when they came into Leyte wore dimethyl phthalate impregnated clothing and it has been suggested that the low incidence of cases in that Island during combat was in part due to this precaution, considering the extent to which the disease was encountered across the Bay in Samar where minimum initial anti-mite clothing protection was practiced. However, in view of the lack of any reported re-impregnation after landing, and the known failure of the repellent used to protect after the first laundering, it appears doubtful to us if this factor was much concerned in the low rate on Leyte.

On Samar, a drop in incidence in February in spite of increasing location of military units, probably reflects a combination of active clearing both by hand, flame throwers (see Fig. 2) and heavy machinery, plus increasing resort to clothing treatment as the outbreak got under way. A few of the patients questioned during sporadic appearance of the disease in subsequent months had not been wearing treated clothing, a practice followed optionally by certain units in the later period. The environment here was chiefly neglected coconut plantations (Fig. 1).

Similar practices were also intensified on Mindoro after the appearance of scrub typhus. Impregnation of uniforms was not accomplished until after the epidemic was under way, but special restrictive measures regarding exposure were applied by the Bomb Wing Surgeon immediately to each of the three major foci in the area as soon as they became apparent. The foci here were chiefly secondary growths of coarse "talahib" grass (*Saccharum spontaneum*) in abandoned cane fields (Figs. 3 & 4).

One of the divisions (the 24th) with experience of infection on Mindoro was later placed in combat on Mindanao where they practiced the wearing of anti-mite clothing initially at least. Again it was suggested without possibility of verification that they failed to encounter more than one case as a result of these precautions on an island where the Japanese previously had reported it.

We may reiterate here what has been discussed before (Kohls et al, 1945), anti-typhus measures during military operations comprise two practical measures (1) mite reduction by manipulation of the environment of the vector, and (2) personal protection which includes the use of so-called miticides in clothing. The first is practicable when stationary installations are projected, but the second is too frequently the only measure that is available during initial operations, and under patrol and combat conditions. These measures are fully discussed in appropriate War Department literature based on previous experience.

SUMMARY

Five species of larval mites of the genus *Trombicula* have been taken on field rats in various of the Philippine Islands during surveys in connection with occurrence of scrub typhus in American troops. These were *akamushi, deliensis, wichmanni, acuscutellaris* and *bodensis* of which the first 4 are known to attack man and the first 2 are demonstrated vectors. Other rat-infesting species taken comprised 3 *Ascoschöngastia,* viz., *indica, philippensis* and *kohlsi,* and *Heaslipia gateri.*

Comment is made upon the variation in the *akamushi-deliensis* complex as found in certain areas which complicates systematic assignment.

Trombicula wichmanni was taken on man in northern Luzon, and is considered by habits and wide distribution in the Archipelago to be a potential incidental vector of scrub typhus.

Trombicula scincoides is a common parasite of lizards and skinks in the Philippines but is not known to be a parasite of man or other warm-blooded animals. An undescribed species of *Trombicula* was found on skinks on Mindoro.

Anti-mite measures during certain military operations are briefly reviewed.

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RESEARCH NOTES

OCCURRENCE OF *EIMERIA SPINOSA* IN SWINE RAISED IN HAWAII

In 1931 Henry (Univ. Calif. Publ. Zool. **36**: 115) described a new species of coccidium, *Eimeria spinosa,* obtained from the caecal contents of a normal pig from California and from a pig which had died upon arrival from Minnesota. The caecum of the latter animal showed lesions as found in swine enteritis. Henry believed that the distribution of this coccidium, which had an unusual spinous cyst wall, was limited since it had not been previously described by others.

The writer is in accord with Henry in that *E. spinosa* is probably not widely distributed, and previous to this report the writer had not noted these forms of coccidia in the examination of several hundred pigs on continental United States and in the Territory of Hawaii. The species of coccidia which are most commonly noted in swine of the latter area are *E. debliecki* and *E. scabra.* Recently, in connection with a study of swine coccidiosis, fresh voided feces of an apparently normal pig raised in the environs of Honolulu showed a large number of coccidia which were diagnosed as *E. spinosa.* The cysts measured 18.5 to 22.2 μ in length and 14.8 to 18.5 μ in width; they were brown in color and the wall was covered with numerous small spines. When cultured in 1 per cent potassium bichromate and kept at room temperature (about 26° C), evidence of sporulation was noted on the ninth day.

The present report is of interest because it records further finding of an apparently uncommon species of coccidium infecting swine and points out a new geographical distribution. As far as the writer is aware outside of the report by Henry, this species has not been recorded as found by others.

Preserved material containing coccidia reported in this paper has been submitted to the U. S. National Museum Collection.

The writer is indebted to Mr. John T. Lucker of the U. S. Department of Agriculture for looking up references regarding reports on the distribution of *E. spinosa* in the Index Catalogue of Medical and Veterinary Zoology at Washington, D. C.—Joseph E. Alicata, *University of Hawaii Agricultural Experiment Station, Honolulu, Hawaii.*

INCIDENCE OF LUNGWORMS AND GASTROINTESTINAL PARASITES IN COPENHAGEN CATS

The lungs of 250 cats from Copenhagen and surroundings were searched for helminth parasites. Two cats showed invasion of both *Aelurostrongylus abstrusus* (Railliet, 1898) Cameron, 1927, and *Capillaria aerophila* (Creplin, 1839) Travassos, 1915; 1 cat contained *Ae. abstrusus* alone and 2 cats *C. aerophila.* Pathological changes were seen in the lung tissue of the 3 cats infested with *Ae. abstrusus* while purulent bronchitis occurred in 2 of the 4 cats infested with *C. aerophila.*

Of these cats 130 were examined for the presence of *Ollulanus tricuspis,* Leuckart, 1865, by microscopic examination of scrapings from the stomach wall. The faeces of these cats were examined by the brine flotation method for the presence of nematode eggs and of coccidia oöcysts. In 100 cats, the whole intestinal tract was opened and investigated for parasites. A microscopic search for small trematodes in scrapings from the intestinal lining, however, was made in only the 20 last cats.

Nine species of parasites were met with in the gastrointestinal tract, *viz.,* 4 nematodes, 1 cestode, 2 trematodes, and 2 coccidia. *O. tricuspis* occurred in the stomachs of 13 of 130 cats; *Capillaria putorii* (Rudolphi, 1819) Travassos, 1915, in the stomach of 1 of 130 cats; *Ancylostoma caninum* Ercolani, 1859, in the small intestines of 2 of 130 cats; *Toxocara cati* (Schrank, 1788) Brumpt, 1927, in the stomachs and small intestines of 67 of 130 cats; *Taenia taeniaeformis* (Batsch, 1786) Wolffhügel, 1911, in the small intestines of 7 of 100 cats; *Cryptocotyle lingua* (Creplin, 1825) Fischoeder, 1903, in the small intestines of 6 of 20 cats; and *Cryptocotyle concava* (Creplin, 1825) Fischoeder, 1903, in the small intestine of 1 of 20 cats. The oöcysts of *Isospora rivolta* (Grassi, 1879) Wenyon, 1923, were found 4 times and the oöcysts of *Isospora felis* Wenyon, 1923, 7 times in the faeces of 130 cats. Nematode larvae of unknown origin were traced in the mucus from the stomach wall of 1 cat. No parasites were found in the urinary bladders of 100 cats; nor was *Trichinella spiralis* seen in the diaphragms of these animals.

Comparison of our results with Krabbe's findings (1866, Recherches helminthol. en Danemark et en Islande, Paris) in 100 Copenhagen cats shows that the tapeworm *Dipylidium caninum,* which at that time with an incidence of 57 per cent was the most frequent parasite of cats, has apparently disappeared from this host, while *Taenia taeniaeformis* and *Toxocara cati* still have about the same prevalence as in 1866. A similar percentage of these two parasites was also seen

by Schmit-Jensen (1921, D. kgl. Veterinær-og Landbohøjskoles Aarskr., Copenhagen, p. 100), who examined 99 Copenhagen cats in 1918 and found 11 per cent of them infested with *Dipylidium caninum*.

The spontaneous occurrence of *Cryptocotyle lingua* in cats is new to science, though *Stunkard* (1930, J. Morph. Physiol. **50**: 143–183) was able to obtain transient experimental infection in this host species. Also, the flukes that we recovered from cats, spontaneously infected, were obviously smaller in size and contained fewer eggs than those recovered from dogs.

Aelurostrongylus abstrusus, Ollulanus tricuspis, Capillaria putorii, Ancylostoma caninum, and *Cryptocotyle concava* are recorded for the first time from Denmark.—N. O. Christensen, Sv. J. Olsen, and Hans Roth, *Dept. of Special Pathology and Therapy (Chief: Prof. H. C. Bendixen) and Dept. of Pathological Anatomy (Chief: Prof. A. F. Følger), The Royal Veterinary and Agricultural College, Copenhagen, Denmark.*

AMERICAN SOCIETY OF PARASITOLOGISTS

Thirty-Fifth Council Meeting, St. Louis, Missouri

March 28, 1946

The meeting of the Council of the American Society of Parasitologists was called to order by President A. C. Chandler at 7:00 PM, March 28, 1946, in Jefferson Hotel, St. Louis, Missouri. Past-Presidents J. E. Ackert and W. W. Cort and the following members of Council were present: D. L. Augustine, R. M. Cable, G. R. Coatney, J. T. Culbertson, A. C. Chandler, C. G. Huff, H. W. Manter, G. F. Otto, E. W. Price, R. M. Stabler, H. W. Stunkard, and W. H. Wright.

I. Reports of Officers

1. *Secretary (J. T. Culbertson):* As of March 1, 1946, there were 537 active members of the Society in good standing, of whom 478 lived within and 59 lived outside continental United States. Seventy-seven persons were elected to membership since the last meeting of Council (December 16, 1944), of whom 65 lived within and 12 lived outside continental United States. Three members died during the year: C. M. Africa, Professor of Parasitology, Institute of Hygiene, Manila, Philippine Islands; C. R. Hamilton, 39th Malaria Survey Unit, Army of the United States; and H. B. Ward, Emeritus Professor of Zoology, University of Illinois. The circumstances of the death of Dr. Africa at the hands of Japanese soldiers were related by W. H. Wright, who had inquired during a recent visit to Manila. Dr. Wright stated that Dr. Africa's untimely death represented a great loss to parasitology. Dr. H. W. Stunkard moved that Drs. Wright and E. W. Price prepare a fitting memorial tribute to Dr. Africa to be transmitted to the family of the deceased and to be published in the Journal of Parasitology. The motion carried.

Council voted to accept the Secretary's report and to place it on file.

2. *Treasurer (R. M. Stabler):* The Treasurer's report for the fiscal year 1945 (Dec. 2, 1944, to March 1, 1946) follows:

Receipts

Balance on hand Dec. 2, 1944			$4085.21
Collected during current year:			
502 member dues applying 1945	$2003.44		
410 member dues applying 1946	1633.76		
16 member dues applying after 1946	50.00		
40 dues in arrears	216.00		
11 applicants	44.00		
		$3947.20	
457½ subscriptions to Volume 31	2095.95		
445 subscriptions to Volume 32	2012.07		
12 subscriptions after Volume 32	55.75		
		4163.77	
Back volumes, numbers, and portraits	537.40		
Advertisements	119.45		
Author's charges	181.50		
Quindex	165.40		
Interest on savings account	15.96		
Miscellaneous	56.85		
		1076.56	
			9187.53
			13272.74

Debits

Printing and handling Journal		
Volume 30	$ 989.96	
Volume 31	2636.52	
		$3626.48

Expenses in office Chr. Ed. Com.	318.32		
Expenses in office Secretary	238.54		
Expenses in office Treasurer	578.06		
Expenses in office Cust. Bk. Nos.	43.28		
		1178.20	
Printing Back Numbers of Journal	599.93		
Printing Portraits	2.25		
		602.18	
			$5406.86
Balance on hand March 1, 1946			7865.88
			13272.74

Audited and found correct, March 29, 1946.
Auditing Committee: (signed) G. F. Otto and
(signed) G. R. Coatney.

Following motion by E. W. Price, Council voted to adopt December 1 to December 1 as the fiscal year for the Society henceforth. Upon motion, the Treasurer's report was accepted, subject to audit, and placed on file.

2. *Other Officers (President A. C. Chandler):* Dr. Chandler reported on his correspondence with Mr. W. A. McCoy of the United States Civil Service Commission, relating to the substitution of experience for formal education in fulfilling requirements for civil service appointments. Dr. Chandler pointed out that at the present time the position of "parasitologist" was not on the special list of professions for which formal training was required.

II. Reports of Committees

1. *Custodian of Princeton Secretarial Fund (N. R. Stoll):* A summary of the report follows:

	"Year" ending Mar. 11, 1946	"Year" ending Sept. 7, 1944
Savings bank balance beginning of year	$ 543.45	$ 538.55
Interest earned	9.43	4.90
Deposit, U. S. Savings Bonds maturing July, 1945	500.00	
Savings bank balance end of year	1052.88	543.45
Value, U. S. Savings Bonds maturing July 1945		480.00
Total	1052.88	1023.45
Total one "year" earlier	1023.45	998.55
Gain, current year	29.43	24.90

Upon motion, the report was accepted, subject to audit, and placed on file.

2. *Chairman of Editorial Committee (H. W. Stunkard):* Volume 31 of the Journal of Parasitology as published comprises the report of the Editorial Committee. The format and editorial policy of previous years have been continued. In recent months, manuscripts have been received in increasing numbers so that Volume 32 will probably require 600 pages. It is hoped that, as labor problems are worked out by the Press, issues of the Journal will appear more promptly. At the present time, approximately six months must elapse between receipt of manuscripts and their appearance in the Journal.

The cost of printing the Journal has increased during the year, although a direct comparison with previous years is difficult because additional copies are now being printed. Fortunately, the American Foundation for Tropical Medicine, Inc., has granted $1000.00 to the Journal, this sum to be paid when an earlier grant of the same amount has been expended, perhaps late in 1946.

The Committee on Aid to Libraries in War Areas has continued its subscription, purchasing 65 sets of each issue of Volume 31 for post-war distribution, at the special price formerly fixed. There are at present 23 exchanges with other journals. It is recommended that this number be increased.

During the war years, books and monographs received for review were listed in the December number of the Journal together with relevant information but without critical review. An opinion was requested from Council on the advisability of printing such reviews. An opinion was also requested from Council as to editorial policy with respect to lengths of papers and charges to authors for manuscripts submitted to the Journal for publication.

Upon motion by J. E. Ackert, seconded by G. F. Otto, the report was accepted, with a vote of thanks to the Editorial Committee.

G. F. Otto moved that, in exchanges, Journal be sent first to abstracting journals and to countries without subscribers. Beyond these limits, the exchange list was to be left to the discretion of the Editor. Motion passed.

Following discussion on the advisability of printing book reviews in the Journal, R. M. Stabler moved that the present policy of printing announcements of books without review be continued. Motion carried.

J. E. Ackert moved that the temporary discretionary powers now vested in the Editorial Committee as to decision on length of articles for the Journal and charges to authors be extended as permanent policy. Motion carried.

W. W. Cort stated that back numbers of the Journal which were in the late H. B. Ward's estate had been received in Baltimore. Dr. Cort moved, seconded by J. E. Ackert, that the Treasurer be authorized to pay for packing and shipping charges for these numbers. Motion passed. Upon motion, Council voted to send a letter of thanks to Miss Charlotte Ward for her generous gift to the Society.

Dr. Stunkard pointed out that objection had been raised by the postmaster in Lancaster, Pennsylvania (Science Press), to including the Journal Supplement at periodical rate. Council voted to continue printing the Supplement separate from the regular issue even at the cost of additional postage charges.

Dr. Stunkard moved that acknowledgment of its generous gift be made to the American Foundation for Tropical Medicine, Inc. Motion carried.

3. *Auditing Committee (G. F. Otto and G. R. Coatney):* The reports of the Treasurer and of the Custodian of the Princeton Secretarial Fund were certified as correct.

4. *Nomenclature Committee (E. W. Price):* The report dealt with authorship of combinations of zoological names, a request having come to the Committee for an opinion on the authorship of the combination *Inermicapsifer cubensis,* a cestode parasite of man in Cuba. Upon motion by G. F. Otto, Council voted to accept the report for publication in the Journal.

5. *Other Committees:* No reports were received from the Committee on Avian Malaria or the Committee on Common Names.

III. Reports of Representatives

1. *To Council of A.A.A.S. (J. E. Ackert and H. J. Van Cleave):* At its meeting on March 27, 1946, the Council of A.A.A.S. voted to adopt January 1 to December 31 as the fiscal year of A.A.A.S., and to adopt the present meeting as the official meeting for 1945. The building fund for the new home of A.A.A.S. contains at present $52,000.00. It is likely that a large block of funds will be added to this sum in a year or so. The A.A.A.S. Council voted to call a general meeting of A.A.A.S. in Boston, Massachusetts, in December, 1946. Upon motion, the report was accepted and placed on file.

2. *To Council of U.A.B.S. (A. C. Walton and A. O. Foster):* President Robert Chambers of U.A.B.S. requested that representatives to the Council hereafter be appointed for three years. Frank A. Brown, Secretary of U.A.B.S., advised that at a joint meeting on March 28, 1946, in St. Louis, steps would be taken to amalgamate the Union and the American Biological Society. Upon motion, the report was accepted and placed on file. Upon motion by E. W. Price, Council voted that henceforth the tenure of representatives to the Council of U.A.B.S. should be for three years.

3. *Other Representatives:* No report was received from W. W. Cort, representative to the Committee on Planning for an International Congress of Tropical Medicine because the Committee had not yet convened. No report was received from W. H. Wright, representative to the National Research Council, Division of Biology and Agriculture, for the reason that he was out of the country when the annual meeting of the organization was held.

IV. New Business

1. *Election of New Members:* The following persons were elected to active membership in the Society: A. M. Griffin, Bacteriology Department, George Washington University, Washington, D. C.; D. L. Kingsbury, 20 Nelson St., Framingham, Massachusetts; Wendell H. Krull, Colorado State College, Ft. Collins, Colorado; Leon G. Lungstrom, 206 S. Cedar St., Lindsborg, Kansas; Marjorie Jean Raecke, Zoological Division, U. S. Bureau of Animal Industry, Beltsville, Maryland; Bernard B. Riedel, Zoology Department, Kansas State College, Manhattan, Kansas.

Following a discussion of requirements for membership in the Society, W. H. Wright moved that "with suitable educational qualification" be inserted in the clause on qualifications for membership in the Society's constitution. The motion carried and the change will be presented for consideration by the entire Society at the next general business meeting.

2. *Plans for next meeting:* Upon motion by G. F. Otto, Council voted to meet as an affiliate of A.A.A.S. in Boston, Massachusetts, during December, 1946, the precise date to be determined later. Motion carried.

3. *Contribution to U.A.B.S., Biological Abstracts, and the International Committee on Zoological Nomenclature:* Upon motion, Council voted to table all requests for contributions by this Society.

4. *Nominations, Elections, and Appointments to Society Offices:* a. The following persons were nominated by Council to the designated Society offices: President for 1946, N. R. Stoll; Vice-President for 1946, H. Kirby, Jr.; Secretary for 1946 and 1947, J. T. Culbertson; Councillors-at-Large through 1949, W. T. M. Cameron and W. H. Wright.

b. The following persons were elected by Council to the Editorial Board of the Journal of Parasitology through 1949: R. M. Cable, W. W. Cort, and L. E. Rozeboom.

c. The following persons were appointed by President Chandler to be representatives for 1946 to the Council of A.A.A.S.: G. R. Coatney and W. H. Wright. Dr. Chandler appointed the following persons to be representatives to the Council of U.A.B.S. through 1948: D. L. Augustine and R. M. Stabler.

d. Dr. Chandler appointed H. W. Manter to the Nomenclature Committee in place of B. G. Chitwood. No other changes were made in standing committees.

It was voted to adjourn at 11:20 PM.

Twentieth Annual Business Meeting, Saint Louis, Missouri March 29, 1946

The general business meeting of the Society was called to order by President A. C. Chandler at 1:20 PM, March 29, 1946, immediately following the annual luncheon in the East Room of Jefferson Hotel, St. Louis. One hundred and twenty-one persons were present.

The reports of the Secretary, Treasurer, and Chairman of the Editorial Committee were presented to the Society, as were the reports of representatives to the Council of A.A.A.S. and U.A.B.S.

The Secretary presented to the Society the names of persons nominated by Council for the Society offices. There were no further nominations and upon motion, duly seconded and carried, the Secretary was instructed to cast one ballot for the nominations as presented. Accordingly, the following persons were elected to the Society offices: President for 1946, N. R. Stoll; Vice-President for 1946, H. Kirby, Jr.; Secretary for 1946 and 1947, J. T. Culbertson; Councillors-at-Large through 1949, W. H. Wright and W. T. M. Cameron.

The Secretary then read the names of the three persons elected by Council to serve through 1949 on the Editorial Board of the Journal, and the President read the names of his appointees, as listed in minutes of the Council meeting.

Dr. R. F. Nigrelli moved, and the Society formally approved, the formation of an organization of biologists on the basis of the two following propositions:

(1) To provide a means for executing our public responsibilities as biologists and scientists, so that the freedom essential to the progress of science and public welfare be ensured;

(2) To safeguard the professional interests of biologists and to assist in providing the material means for the promotion of biological research; also to provide such services as may be necessary to facilitate this program.

These propositions had previously been presented to the Society by Dr. A. C. Walton, representative to the Council of U.A.B.S.

A vote of thanks was extended to Dr. H. Tsuchiya for his services as local representative of the Society for the St. Louis meeting.

The Society voted to adjourn at 2:10 PM, to meet again at the A.A.A.S. convention in Boston, Massachusetts, during December, 1946.

Respectfully submitted,
James T. Culbertson, *Secretary.*

REPORT OF THE COMMITTEE ON NOMENCLATURE

Authors of Combinations of Zoological Names

The priority of authorship of combinations of zoological names is not too clearly stated in the International Rules of Zoological Nomenclature, although the implication is clear and usage seems to have established the principle that the author of a combination is that person who first actually made the union of a generic and specific name.

Article 23 of the "Rules" states "When a species is transferred to another than the original genus or the specific name is combined with any other generic name than that with which it was

originally published, the name of the author of the specific name is retained in the notation but placed in parentheses" and "If it is desired to cite the author of the new combination, his name follows the parentheses."

A case in point, which has been referred to the Society's committee on nomenclature for opinion, involves the authorship of the combination *Inermicapsifer cubensis,* a cestode parasite of man in Cuba.

In an abstract published in The Abstracts of Communications of the Third International Congress for Microbiology (1939) Kourí briefly described a tapeworm of man under the name *"Raillientina cubensis,* n. sp." In the (1940) Report of the Proceedings of the Congress, Kourí and Rappaport stated "After studying the first few specimens, we thought that we had found a new species of *Raillietina.* Later, with the kind cooperation of Professor J. G. Baer, study of new material containing scolices caused us to rectify our first impression. According to Professor Baer this species, probably new, must be placed in the genus *Inermicapsifer* Janicki, 1910; subfamily Linstowinae Fuhrmann, 1907; family Anoplocephalidae Fuhrmann, 1907." It may be noted that in this communication the specific name *cubensis* was not mentioned.

Stunkard, in a paper published in 1941 (Rev. Med. Trop., Parasitol. Bacteriol., Clin. y Lab. 6(5 & 6) : 285–288 (1940)) on "Tapeworm infection in the West Indies," made, apparently for the first time (p. 285), the combination *"Inermicapsifer cubensis* (Kourí, 1939)." Later, Kourí (1943) (Ibid. 9(3) : 29–31) and again (1944) (Ibid. 10(5 & 6) : 107–112) refers to *Inermicapsifer cubensis* (Kourí 1939) Kourí 1940, apparently believing the report by Kourí and Rappaport of the conclusion of Prof. Baer, as expressed in the report of the Third International Congress of Microbiology, that the species (earlier reported as *"Raillientina cubensis* n. sp.") belonged in the genus *Inermicapsifer* constituted a transfer of the species in question to that genus.

In view of the facts stated above it is evident that Kourí at no time prior to 1941 actually made the combination *Inermicapsifer cubensis,* and it is our opinion that the proper citation is *Inermicapsifer cubensis* (Kourí, 1939) Stunkard, 1941.

B. G. Chitwood
A. McIntosh
E. W. Price, *Chairman*

APPLICATION FOR HOUSING ACCOMMODATIONS FOR ANNUAL MEETING, BOSTON, MASSACHUSETTS, DECEMBER 26TH–28TH, 1946

All reservations for hotel rooms or other housing accommodations must be filed not later than December 10, 1946, with

AAAS Housing Bureau,
Convention Bureau, Chamber of Commerce,
80 Federal Street, Boston 10, Massachusetts.

The name of each person must be listed for whom reservation is requested, together with the type of accommodation desired. The definite dates and hours of arrival and departure must be submitted for each guest. The Statler Hotel will be the official headquarters of the American Society of Parasitologists. The rate for a single room at the Statler Hotel is $3.85 to $5.50 per day. The rates for single rooms at other hotels nearby follow:

Lincolnshire	$3.30 to $4.40	Parker House	$3.85 to $4.40
Commonwealth	2.50	Bradford	3.30 to 3.85
Bellevue	3.30 to 4.40	Copley Plaza	4.40 to 7.70

The housing bureau states that rooms will be assigned and confirmed in order of receipt of reservation. All applications should indicate first, second, and third choices of hotel.

J. T. Culbertson, *Secretary.*